Distribution of Hexachlorobenzene Concentrations in Spruce Needle Samples across Alaska

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Abstract

The global distribution of persistent organic pollutants has initiated considerable effort towards understanding long range atmospheric transport and partitioning of these potentially damaging compounds. Apparent latitude dependent concentration gradients of organic pollutants in otherwise pristine environments has given rise to a global fractionation model, coined the cold finger effect. According to the cold finger theory, semi-volatile persistent organic pollutant will show a preference for partitioning from the atmosphere to the ground and vegetation at northern latitudes. Here we present a study of hexachlorobenzene in spruce needle samples across Alaska, which offers a large range of climates, from its southern coastal rain forests to the northern arctic. The large variation in climate across Alaska should result in a measurable latitude dependent concentration gradient for HCB, if the cold finger effect is being realized. Spruce needle samples were extracted, cleaned, and analyzed by GC/MS. According to principle component regression analysis, HCB concentrations in all the spruce needle samples across Alaska show a strong positive correlation with lipid content of the needles. The HCB concentrations also show two distinct latitude trends. The spruce needle samples taken from the coast to approximately 63^0 north show relatively high HCB concentrations and a possible negative correlation with latitude. The samples between 63^0 and 68^0 north show a definite positive correlation between HCB concentration and latitude, which is consistent with the cold finger effect.

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Chapter 1
Introduction

Widespread use and long range atmospheric transport have distributed many persistent organic pollutants throughout the world (1). Potentially damaging compounds, such as pesticides, occur even in areas that show no history of the compounds use. The reality of long-range atmospheric transport was clearly demonstrated by D. A. Peel in 1974 (2). Peel and colleagues collected snow samples 450 Km inland of the coast of Antarctica. The snow samples represented the winters of 1965-1969. DDT and DDE were detected in all samples, with a concentration range of 0.1 to 3 picograms per gram of water. This study soundly illustrates long-range atmospheric transport, as the Antarctic continent shows no history of pesticide use.

The presence of such pollutants in otherwise pristine environments has initiated considerable effort towards understanding the nature of their atmospheric transport and partitioning processes. In 1974 C. Rappe proposed the rule of the cold wall, stating that organic pollutants may evaporate from relatively warm regions and condense in lower temperature regions (3).

In 1993 Wania and Mackay further proposed a global fractionation model. According to this model pollutants become latitudinally fractionated based on the pollutants vapor pressure, with higher vapor pressure corresponding to condensation at higher latitudes (4). Numerous studies have since suggested a persistent organic pollutant latitude concentration gradient (5,6).

The theory of global fractionation of organic pollutants has since been coined the cold-finger effect. Wania and Mackay define four global mobility classes for persistent organic pollutants, based on their vapor pressure and octanol-air partitioning coefficients (6). The four classes are low mobility, relatively low mobility, relatively high mobility, and high mobility. The low mobility class is expected to show no latitude trend, as these compounds exhibit low vapor pressure, little mobility, and are dominated by permanent retention and degradation. The high mobility class also shows no latitude trend, as these compounds tend not to condense significantly at global ambient temperatures. A latitude concentration gradient is expected for the relatively low and relatively high mobility classes, with preferred condensation at mid-latitudes and high latitudes respectively (7).

Recent research has shown considerable interest in using plants as passive air samplers (8-10). Legitimate use of plants as air samplers requires an understanding of the mode of analyte uptake.

Studies have demonstrated that hydrophilic pollutants enter the plant primarily through the roots, thereafter being transported to the leaves and other parts of the plant (11-13). Therefore, the concentration of hydrophilic pollutants in plants will reflect concentrations in the soil resulting from atmospheric deposition, presuming the absence of other sources of contamination. Juuti S. employed pine needles in this regard to successfully identify two pulp mills in Finland as sources of trichloroacetic acid (14).

Lipophilic pollutants enter the plant leaves directly by vapor phase transition between the atmosphere and the waxy cuticle on the leaf surface, and by the absorption of aerosol particles by the waxy cuticle, with other modes of analyte uptake being insignificant (11-13). Therefore, the concentration of lipophilic pollutants in plants reflects both atmospheric concentrations and the degree of analyte partitioning from the atmosphere to the plant. This makes plants prime candidates for investigating cold finger behavior of lipophilic persistent organic pollutants.

The primary goal of this investigation is to look for possible latitude concentration gradients for chlorinated pesticides in spruce needle samples across Alaska. If the cold finger effect exists it should be strongly pronounced in Alaska because of the strong temperature gradient across the state. The analysis centers on pesticides in the relatively high mobility class, as these compounds are expected to be more prevalent in the northern latitudes of Alaska, with concentrations increasing with latitude.

Secondary to investigating the cold-finger effect is a through effort to develop experimental methods specific to this analysis. Often times during environmental analyses samples are prepared and analyzed using well known techniques, applied in a cookbook approach. This approach will often give acceptable results, however it may be inefficient in terms of labor and resources, and opportunities may be lost. Therefore, before analyzing samples collected across Alaska we developed efficient sample preparation techniques specific to our needs.

Hexachlorobenzene was the sole chlorinated pesticide found in the spruce needle samples across Alaska. The HCB levels of these samples, and the range of the HCB levels, resemble that seen in other studies of chlorinated compounds in plant samples (1-12). A principle component regression analysis identified a strong positive correlation between HCB levels and sample lipid content, along with two latitude trends. Samples from the southern coast to the south side of the Alaska range (approximately 60^0 to 63^0 north) gave relatively high HCB levels and a negative correlation with latitude. However, sample taken between 63^0 and 68^0 north showed a positive correlation with latitude, consistent with the cold finger theory.

Chapter 2

Experimental Procedures and Method Development

2.1 Materials and Sample Sites

Solvents were of GC grade and were supplied by EM Science. Aldrich supplied silica gel (40μm average diameter). Hewlett Packard model 5890 GC and model 5972 Mass Selective Detector were used for the GC/MS analysis. Supelco supplied standards and internal standards, each of which is described below:

EPA 8080/8270 Pesticide Surrogate Mix Catalog # 4-7903
Dibutyl Chlorendate
2,4,5,6-Tetrachloro-m-Xylene

Chlordane Catalog # 4-0089
α/γ - Chlordane

Hexachlorobenzene Catalog # 4-0008
HCB

Dieldrin Catalog # 4-0088
Dieldrin

TCL Pesticide Mix Catalog # 4-8913
Aldrin
Endrin
Dieldrin
Endosulfan I / II
Endosulfan Sulfate
Endrin Aldehyde / Ketone
Heptachlor
Epoxyheptachlor
HCH (α,β,γ,δ)
Methoxtchlor
4,4' DDD / DDT / DDE

Thirty six duplicate samples were taken along the highways during the last week of March 1997. Branches were removed from the trees, at approximately four feet from the ground, cut up, and stored in plastic bags. Tree samples were kept below freezing temperature during collection and storage. Figure 1 shows the locations of the sample sites, and descriptions for each are listed in appendix 1.

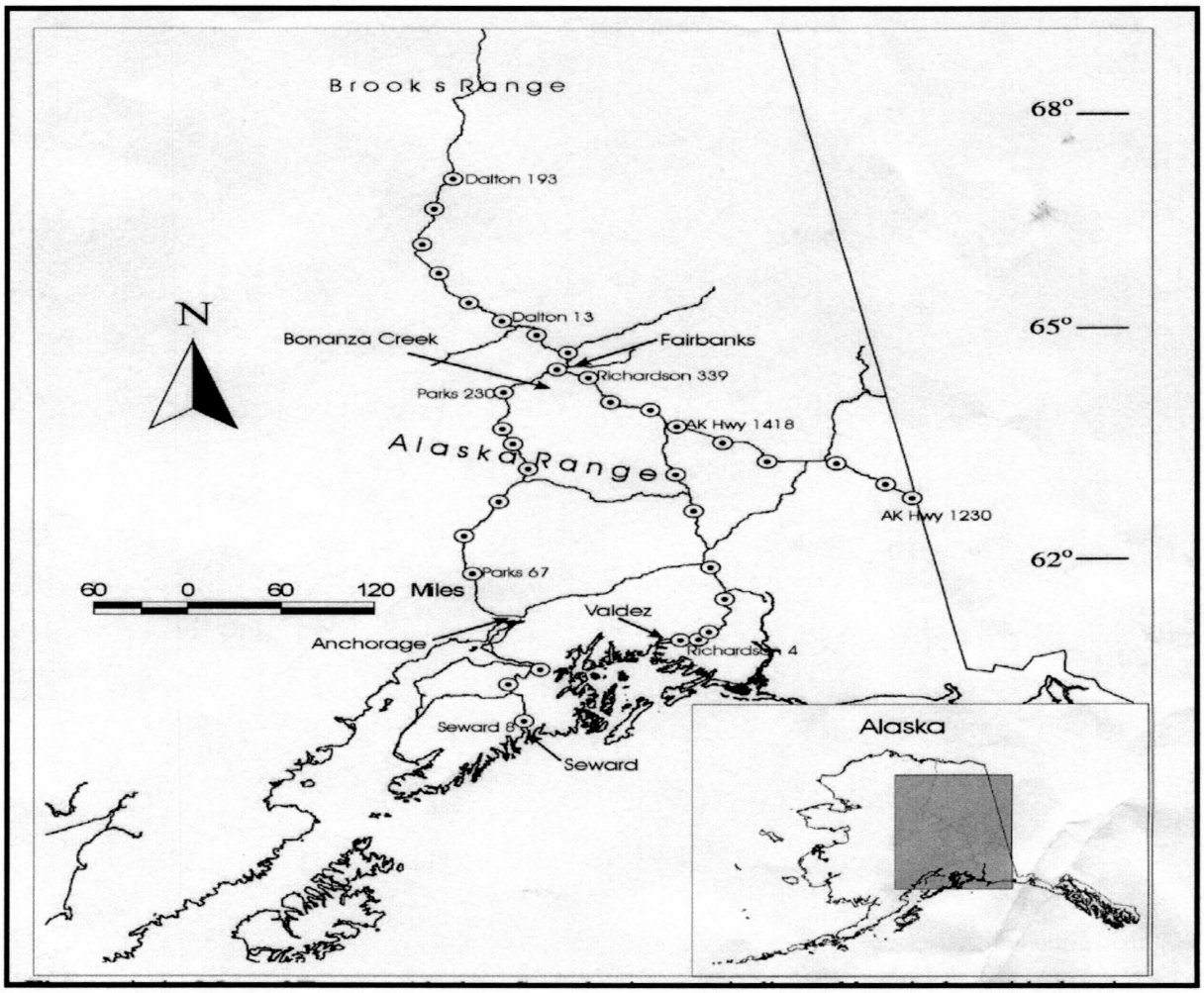

Figure 1 Locations of the Sample Sites.

In addition to the above samples, spruce needles were collected for method development from the Bonanza Creek forest research area, approximately 20 miles southwest of Fairbanks near the Tannana River.

2.2 Sample Scheme and Quality Control

Two trees were sampled and both were analyzed for each sample site. For all samples 250 μl of 250 ppb internal standard (2,4,5,6,tetrachloro-m-xylene) was added before extraction. Sample sites were chosen for complete duplicate analysis and for standard additions, as described below. The standard addition samples were spiked, before extraction, with 250 μl of a standard mixture made with the pesticide standards listed in section 2.1. Final concentrations for the components of the standard mixture were 250 ppb.

Richardson Highway

Mile Post	Spike	Duplicate
16	X	
31		X
166	X	
207		X
309	X	
339		X

Dalton Highway

Mile Post	Spike	Duplicate
13		X
50	X	
193	X	

Parks Highway

Mile Post	Spike	Duplicate
107	X	
147		X
230	X	
270		X

Alaska Highway

Mile Post	Spike	Duplicate
1230	X	
1301		X
1417	X	

Seward Highway

Mile Post	Spike	Duplicate
8		X
45	X	
75		X

Elliot Highway

Mile Post	Spike	Duplicate
9		X
45	X	

All glassware used for sample work up was washed between uses with soap and water, and then baked at 250° C for at least two hours.

Extractions were done in groups of six, with one method blank per group. The method blanks were processed using all the steps of the sample work up, however no needles were used. The developments of the sample preparation and analysis techniques follow in individual sections.

2.3 GC/MS Analysis

A GC method for pesticides from Supelco was used to initiate the development of the GC/MS method. Conditions for this GC method are listed in Table 1, and total ion mode was used for the mass selective detector.

Table 1 Parameters for the Initial GC/MS Analysis

Column	PTE-5, 30 m x 0.25 mm ID, 0.25 μm film thickness.
Oven	60° C for 3 minutes, 25° C/minute to 180° C, 4° c/minute to 300° C, hold for 5 minutes.
Carrier Gas	Helium (1 ml/minute)
Analyte	10 ppm standards + internal standard in hexane.
Injection Parameters	Splitless mode. No pressure programming. Purge after 1 minute.
Injection Volume	1 μl

The above temperature program was modified to resolve hexachlorobenzene and α-hexachlorocyclohexane. The revised temperature program is listed below:

Oven	100° C for 3 minutes, 3° C/minute to 226° C, 30° C/minute to 280° C, hold for 5 minutes.

The resulting retention times and major ions from the total ion chromatograms were used to develop windows for selected ion monitoring. The three m/z values used for each compound are listed in Table 2.

Table 2 Selected Ion Monitoring m/z Values.

Compound	**m/z Values**
2,4,5,6-Tetrachloro-m-Xylene	207,209,244
HCB	142, 284, 286
Aldrin	66, 263, 265
Endrin	81, 263, 265
Dieldrin	77, 79, 82
Endosulfan I	195, 239, 241
Endosulfan II	159,195,237
Endosulfan Sulfate	229, 272, 274
Endrin Aldehyde	67, 209, 245
Endrin Ketone	66, 67, 101
Heptachlor	100, 272, 274
Epoxyheptachlor	81, 357, 359
HCH (α,β,γ,δ)	181, 183, 219
Methoxtchlor	114, 227, 228
4,4' DDD / DDT	165, 235, 237
Chlordane	237,273,275
4,4' DDE	246, 248, 318

An autosampler was employed for the GC/MS analysis of the tree samples. Ten samples, consisting of needle extracts and method blanks, were run for each sequence. Samples and blanks were placed in the autosampler in random order. Standards and hexane blanks were run before and after each set of five samples. After each sequence the GC oven was taken to 280° C and held for two hours.

Single point regression, using the 250 ppb standard/internal standard, was used for the quantitative measure of analyte in samples. The validity of using a single point regression was tested by GC/MS analysis of the standard over a range of concentrations (0, 125, 250, 500, 750, and 1000 ppb).

2.4 Column Chromatography Sample Cleanup

Samples were cleaned by column chromatography, prior to GC/MS analysis. Three investigation of sample cleanup were done with two general goals.

The first investigation was done to determine if silica could give an adequately clean sample for pesticide analysis. Florisil is more commonly used for cleaning samples prior to pesticide analysis. However, in conjunction with the current work Tim Howe wanted to analyze the samples for PAH's, using silica for sample cleanup (15). A single cleanup technique for pesticides and PAH's using silica will be more convenient, as a single sample may be processed and analyzed for both groups of analytes. The parameters used in the first analysis are listed in Table 3.

Table 3 Parameters for the First Investigation of Sample Cleanup.

Glass column	10 mm inside diameter
Stationary phase	(1) 40μm silica, 4.0 grams (2) florisil , 4.0 grams
Sample	100 μl 20 ppm standard/ internal standard
Flow rate	4 – 5 ml / minute
Elution profile	19 ml hexane 19 ml (15:85) methylene chloride:hexane 25 ml (1:1) methylene chloride:hexane
Fraction volume	2.5 ml

The second and third investigations of cleanup technique were keyed toward decreasing complexity. Often chromatography cleanup of pesticides involves multiple solvents or mixtures, resulting in classes of pesticides being well resolved. For this work resolving classes of pesticides during sample cleanup is not necessary because the GC/MS analysis will fully resolve and quantify any of the pesticides in the spruce needles. The second and third investigations involved simplifying the solvent profile and determining if the resulting samples were clean enough for the GC/MS analysis.

The second analysis excluded the 15% methylene chloride fraction. The parameters for the second analysis are listed in Table 4.

Table 4 Parameters for the Second Investigation of Sample Cleanup.

Glass column	10 mm inside diameter
Stationary phase	40 μm silica, 4.0 grams
Sample	100 μl 20 ppm standard/internal standard
Flow rate	1 – 1.5 ml / minute
Elution profile	45 ml hexane 20 ml (1:1) hexane:methylene chloride
Fraction volume	5 ml

The third investigation was performed using the 50% hexane : 50% methylene chloride as the only solvent. Two analyses were done, each differing in the sample being separated. For the first analysis the sample was identical to that listed above. For the second analysis 100 μl of 20 ppm standard/internal standard was used to spike 50 grams of ground needles. This sample was extracted for 6 hours in 150 ml hexane and then filtered. Volume was reduced to approximately 3ml with a rotary evaporator, then evaporated under nitrogen to approximately 250 μl.

Parameters for the third column chromatography investigation are shown in Table 5.

Table 5 Parameters for the third Investigation of Sample Cleanup.

Glass column	10 mm inside diameter
Stationary phase	40 μm silica, 4.0 grams
Sample	(1) 100 μl 20 ppm standard/internal standard (2) 50 grams ground needles + 100 μl 20 ppm standard/internal standard
Flow rate	1 – 1.5 ml / minute
Elution profile	20 ml (1:1) hexane:methylene chloride
Fractions Collected	5 ml

2.5 Extraction Technique

The Bonanza Creek sample site needles were used for the analysis of the extraction technique. The variables of interest were investigated through fractional factorial design, generated with Design Expert 5 software (STAT-EASE Inc, Minneapolis, MN). Two separate factorial analyses were done. The variables for the first factorial analyses are described in Table 6, along with high and low ranges.

Table 6 Parameters for the First Factorial Analysis of Extraction Technique.

Variable	Low Range	High Range
Grinding in Blender	Needles Not Ground	Needles Ground
Drying Time (50° C)	0 Hours	20 Hours
Extraction Temperature	5° C	25° C
Extraction Time	2 Hours	22 Hours
% Acetone (In Hexane)	0 %	20 %
Solvent Volume	100 ml	75 ml
Agitation	Not Stirred	Stirred Continuously

The 1/16 fractional factorial design for the above variables is shown in Table 7. The high and low values for the variables are designated H and L.

Table 7 Design Values for the First Factorial Analysis of Extraction Technique.

Design #	Grinding	Drying (Hours)	Temp. (° C)	Time (Hours)	Acetone (%)	Volume (ml)	Agitation
1	L	L	L	L	H	L	L
2	H	L	L	L	L	L	H
3	L	H	L	H	H	H	H
4	H	H	L	H	L	H	L
5	L	L	H	H	L	H	H
6	H	L	H	H	H	H	L
7	L	H	H	L	L	L	L
8	H	H	H	L	H	L	H

The first factorial analysis used the weights of the nonvolatile extractables, before and after column chromatography, as responses.

The solvent was removed from the needles, using a fine fritted glass filter, then reduced with a rotovap using a tared flask. The difference in weight for the flask estimates the weight of nonvolatile extractables before column chromatography.

For the measure of nonvolatile extractables after column chromatography the above extract was resuspended in approximately 3 ml hexane and cleaned by column chromatography using the following conditions:

Glass Column	10 mm Inside Diameter
Stationary Phase	4.0 g Silica (40μm)
Flow Rate	1-1.5 ml / minute
Solvent	25 ml 1:1 Hexane:Methylene Chloride

The second factorial analysis was done with Soxhlet extraction as a variable, in addition to grinding, extraction time, and percent acetone. The variables, with their high and low values, are shown in Table 8.

Table 8 Parameters for the Second Factorial Analysis of Extraction Technique.

Variable	**Low Range**	**High Range**
Extraction Technique	Static Extraction	Soxhlet
Grinding	Needles Not Ground	Needles Ground
Extraction Time	2 Hours	6 Hours
% Acetone (In Hexane)	0 %	20 %

The full factorial design is shown in Table 9, with high and low values for the variables designated H and L.

Table 9 Design Values for the Second Factorial Analysis of Extraction Technique.

Design Number	Extraction Technique	Grinding	Extraction Time (Hr)	% Acetone (In Hexane)
1	L	L	L	L
2	H	L	L	L
3	L	H	L	L
4	H	H	L	L
5	L	L	H	L
6	H	L	H	L
7	L	H	H	L
8	H	H	H	L
9	L	L	L	H
10	H	L	L	H
11	L	H	L	H
12	H	H	L	H
13	L	L	H	H
14	H	L	H	H
15	L	H	H	H
16	H	H	H	H

For this analysis the extracts were filtered, volumes reduced, and cleaned by column chromatography using the conditions listed above.

After chromatography the samples were reduced by rotovap to 3 ml, then evaporated to approximately 250 µl with nitrogen. GC/MS analysis was performed using the final conditions outlined in section 2.3.

2.6 Analyte Concentration versus Needle Location and Age

An investigation was done to determine the variability of analyte concentration within a single tree. An entire tree, with a trunk diameter of approximately six inches, was cut down in the Bonanza Creek sampling area.

Branches were divided into three groups, top, middle, and bottom. In addition, needles from each group were divided into new growth (growth from the previous summer) and old growth. Duplicate samples were taken for each of these six groups.

For each of the twelve samples, 50 grams of needles were ground and extracted at room temperature in 200 ml hexane for 60 hours. The extract was decanted and the needles washed with 100 ml hexane. The duplicate extracts were prepared and analyzed individually as described below.

The sample extracts were rotary evaporated down to approximately 3 ml and cleaned by column chromatography under the following conditions:

Glass Column	10 mm Inside Diameter
Stationary Phase	40 μm Silica, 4.0 grams
Flow Rate	1 – 1.5 ml / minute
Elution Profile	35 ml Hexane 35 ml (1:1) Hexane:Methylene Chloride

For each sample the column effluent was collected as a single fraction then rotary evaporated to 3 ml, and then evaporated to approximately 250 μl with nitrogen. Analysis was done by GC/MS as described in section 2.3.

2.7 Recovery of Analyte and Internal Standard

An investigation was done to determine percent recovery of analyte during sample work-up. Five groups of samples were processed using different parts of the sample work-up procedures. Groups one through four were 250 µl portions of a 1 ppm standard/internal standard mixture, run in duplicate. Group five used two 10 gram needle samples, one of which was spiked with 250 µl of the 1 ppm standard/internal standard. Table 10 shows descriptions of the samples, the sample treatments, and the possible sources of analyte loss.

Table 10 Description of the Source of Analyte Loss Experiment.

		Sample Treatment			
Group	Sample	Rotovap	Evaporate Dry	Column Chromatography	Possible Source of Loss
1	Standard/Internal Standard	*			Rotovap
2	Standard/Internal Standard	*	*		Rotovap + Evaporating to Dryness
3	Standard/Internal Standard				None (Control)
4	Standard/Internal Standard	*		*	Rotovap + Column Chromatography
5	10 grams Needles + Standard/Internal Standard	*	*	*	All 3 Sources
5	10 grams Needles	*	*	*	All 3 Sources

Sample volumes were reduced under nitrogen to approximately 250 µl. GC/MS analysis was performed using the final conditions listed in section 2.4 above.

2.8 Analysis of GC/MS Results

Following method development, the samples collected across Alaska were processed and analyzed by GC/MS, with replicate and spiked samples as described in section 2.2.

All GC/MS data were scrutinized to remove possible outliers prior to a multivariate analysis. Individual tree samples were removed only if they differed from other samples in the same sample site and neighboring sample sites by 100% or more. This initial screening for outliers was intentionally conservative, as any additional outliers will be identified and removed during the multivariate analysis. Only twelve of the 87 tree samples were removed according to these criteria.

Average temperature and precipitation readings for January, February, and March of 1997 were obtained for thirteen weather stations along the highways of interest. The data are available at www.wrcc.sage.dri.edu/summary/climak.html, supplied by the University of Alaska Fairbanks Geophysical Institute. The average temperature and precipitation for the three months was used in the analysis, with values interpolated between weather stations.

The analysis was done with the multivariate package The Unscrambler (CAMO ASA, Norway). A detailed description of multivariate analysis is beyond the scope of this work. If the reader is unfamiliar with multivariate techniques the text "*Multivariate Analysis in Practice*" by K Esbensen et al is recommended (available through CAMO ASA, www.akses.no/camo/). Values for HCB levels and lipid content were averaged for each sample site. The multivariate analysis involved eleven variables, with categorical variables coded into the analysis as matrices of +1 and −1. Concentration of hexachlorobenzene, nanograms per gram of needle dry weight, was used as the response. The data was analyzed by principle component regression, and the model was validated by leverage correction.

The variables used in the principle component regression analysis, along with the range for each variable, are listed in Table 11.

Table 11 Variables used in the Principle Component Regression Analysis.

Variable	Variable Type	Variable Range
Tree species	Categorical	Sitka spruce, black spruce, or white spruce
Terrain	Categorical	Lowland, muskeg, bottom land, upland, tundra, alpine, or coastal
Closest city	Categorical	Fairbanks or Anchorage
Radial distance to closest city (degrees)	Numerical	0.01 to 3.5
Average temperature (^{0}F)	Numerical	-8.0 to 31.0
Average precipitation (inches)	Numerical	0.1 to 0.54
Elevation (feet)	Numerical	100 to 2800
Latitude (degrees north)	Numerical	60.1 to 67.5
Lipid (g/10 g needles)	Numerical	0.09 to 0.40
[HCB] (ng/g dry weight)	Numerical	1.07 to 3.05

Chapter 3
Results and Discussion

3.1 GC/MS Technique

The revised temperature program, shown in section 2.3, was employed for the sample analysis as it fully resolved hexachlorobenzene and α-hexachlorocyclohexane. The γ and δ hexachlorocylohexane (HCH) co-elute. However this is not a concern, as HCH was not observed in any of the tree samples. A chromatogram of the pesticide standard using this temperature program is shown in Figure 2. The internal standard is 2,4,5,6 tetrachloro-m-xylene.

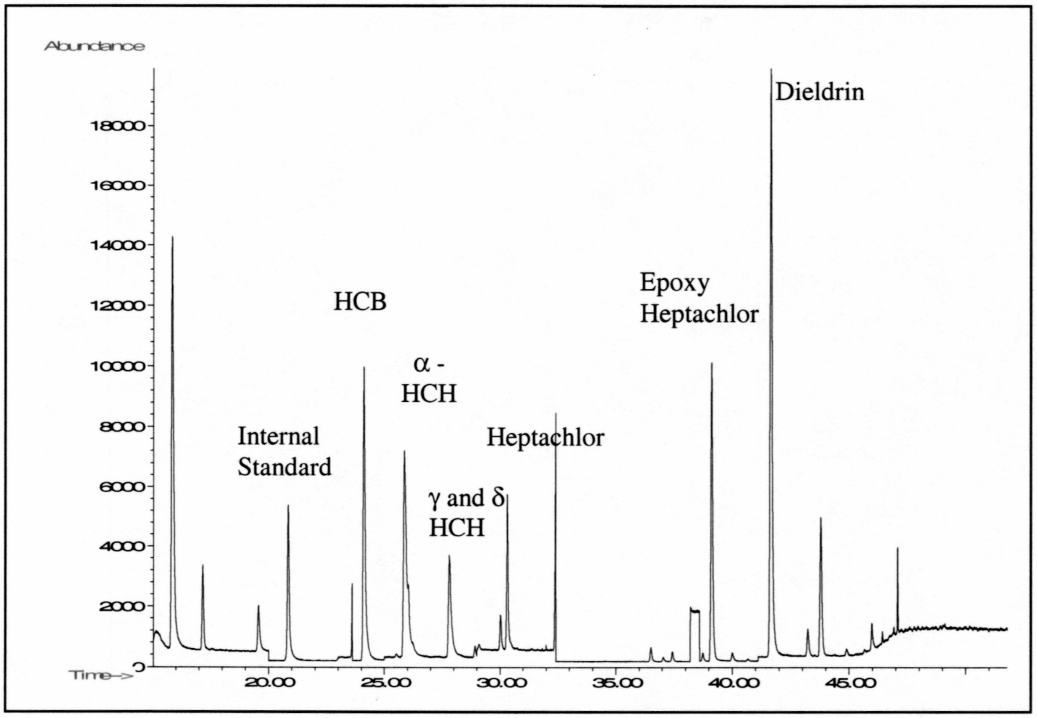

Figure 2 Pesticide Standard Chromatogram using the Final Temperature Program.

Selected ion monitoring was used throughout the analysis to increase sensitivity and selectivity, and to minimize bias due to substances that may co-elute with the analyte. Figure 3 shows a portion of a chromatogram of a tree sample spiked with pesticide standard.

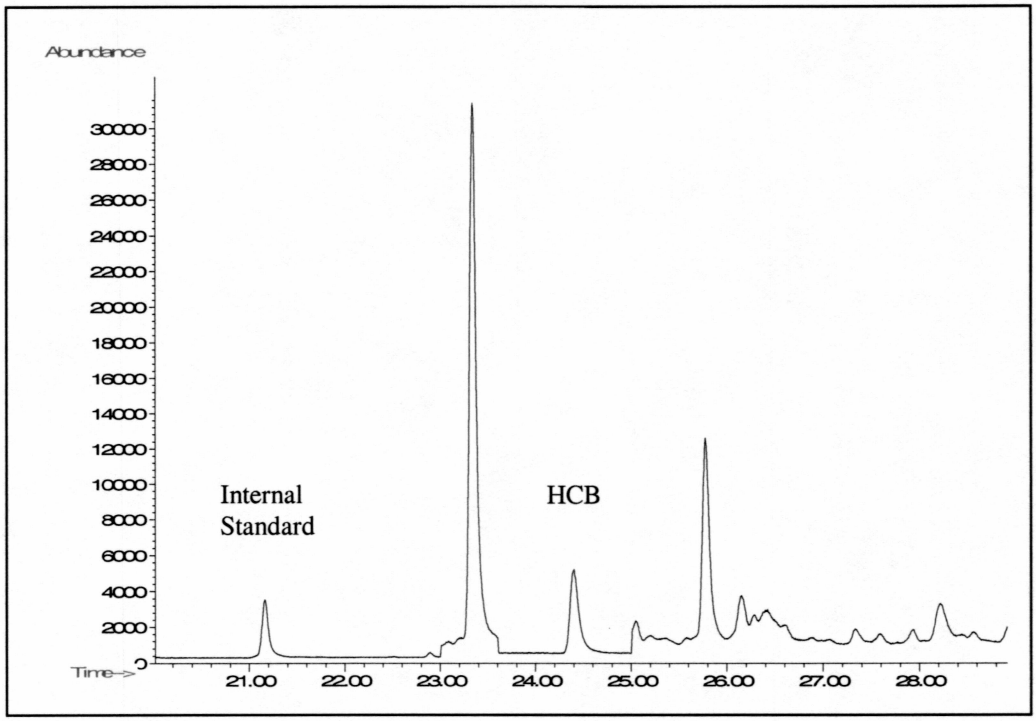

Figure 3 Chromatogram of a Tree Sample Spiked with Pesticide Standard.

During the analysis of the tree samples the sequence order described in section 2.2 was followed. The absence of analyte in the hexane blanks, injected after each standard run, confirmed the absence of carry over from the GC syringe.

During early method development inconsistencies in retention times were observed in standards and tree samples, along with a progressive decline in peak areas for the standards. This was attributed to the relatively dirty tree samples fouling the front of the GC column. Baking the column before and after each sequence solved this problem. This can be seen in the consistency of the HCB and 2,4,5,6 Tetrachloro-m-Xylene peak areas for the standards within each sequence, shown in appendix 2.

All quantitative determinations were based on a single point regression of a 250 ppb standard. The validity of using single point regression was confirmed by the linear relationship between GC/MS peak area and analyte concentration. The peak areas and resulting regressions for four of the analytes, at varying concentrations, are shown below in Table 12 and Figure 4. Other analytes within the standard gave similar results.

Table 12 Peak Area vs. Pesticide Standard Concentration.

Analyte	**0 ppb**	**125 ppb**	**250 ppb**	**500 ppb**	**750 ppb**	**1000 ppb**
HCB	0	$2.6x10^4$	$8.61x10^4$	$1.64x10^5$	$2.39x10^5$	$2.97x10^5$
a-HCH	0	$2.51x10^4$	$4.40x10^4$	$8.96x10^4$	$1.24x10^5$	$1.68x10^5$
DDT	0	$1.63x10^4$	$3.14x10^4$	$7.17x10^4$	$9.65x10^4$	$1.48x10^5$
Endosulfan	0	$6.55x10^3$	$1.19x10^4$	$2.41x10^4$	$3.48x10^4$	$4.68x10^4$

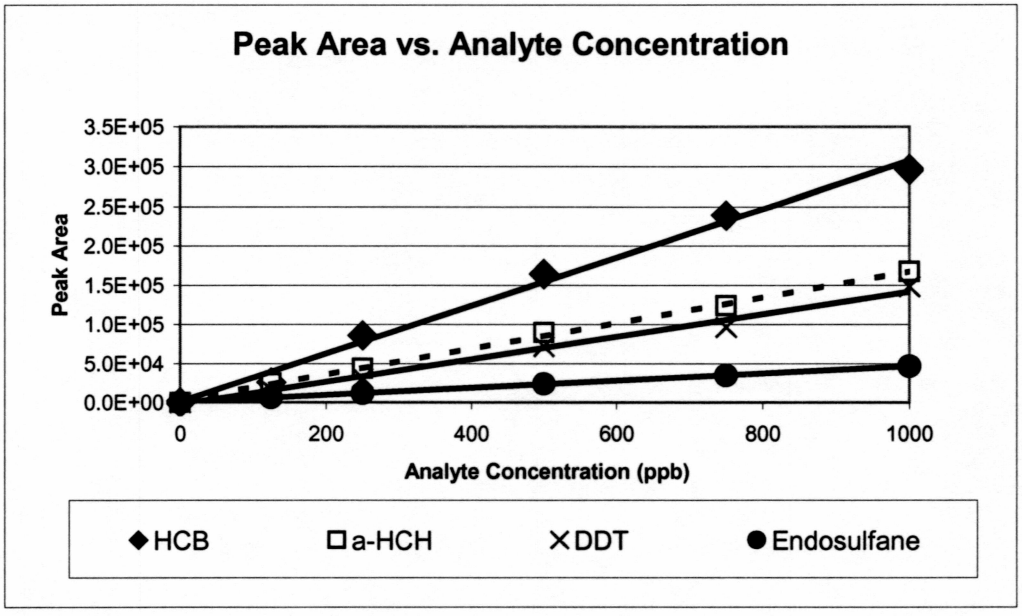

Figure 4 Peak Area vs. Analyte Concentration.

3.2 Sample Clean-up

Tables 13 and 14 show the elution profile of the TCL pesticide mix (described in section 2.1), separated on silica and florisil according to the first set of parameters in section 2.4. An X indicates analyte present in the fraction.

Table 13 Elution Profile for the Florisil Column.

	Fraction Number																				
Pesticide Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Heptachlor		X																				
DDT		X	X																			
d-HCH		X	X	X	X																	
Aldrin		X	X	X	X	X	X															
DDD		X	X	X	X	X	X	X														
DDE		X	X	X	X	X	X	X	X	X	X	X	X	X								
a-HCH						X	X	X	X	X	X											
g-HCH							X	X	X	X	X	X	X									
b-HCH									X	X	X	X	X	X	X							
Endosulfan I										X	X	X	X	X								
Epoxy Heptachlor										X	X	X	X	X								
Endrin										X	X	X	X	X	X	X	X	X	X	X	X	X

Table 14 Elution Profile for the Silica Column.

	Fraction Number																					
Pesticide Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Heptachlor								X														
DDT										X	X	X										
d-HCH																				X	X	
Aldrin					X	X	X															
DDD										X	X	X	X	X	X							
DDE					X	X	X	X	X	X	X	X	X									
a-HCH													X	X	X	X						
g-HCH																X	X	X	X			
b-HCH																		X	X	X		
Endosulfan I																			X	X	X	
Epoxy Heptachlor																		X	X	X		
Endrin																				X	X	X

The silica gel gives higher retention of the analytes, along with narrower bands. This can be seen as each analyte first shows up in a latter fraction for the silica gel, and is present in a smaller number of the fractions. The silica gel was chosen for the remainder of the method development and analysis. The narrower analyte bands are more conducive to separating analytes from interfering compounds in the tree samples.

Shown in Table 15 is the elution profile for the silica gel separation of both standards, without the use of the 15% methylene chloride fraction.

Table 15 Elution Profile Without the 15% Methylene Chloride Fraction.

	Fraction Number											
Pesticide Species	1	2	3	4	5	6	7	8	9	10	11	12
Heptachlor			X									
DDT			X									
d-HCH							X	X	X	X		
Aldrin	X	X										
DDD			X	X								
DDE	X	X										
a-HCH			X	X								
g-HCH				X	X							
b-HCH				X	X	X	X					
Endosulfan I		X	X									
Dieldrin			X	X	X							
Chlordanes		X	X									
HCB	X	X										
Endrin										X	X	X

Removing the 15% methylene chloride fraction gives roughly the same elution order, with tighter elution bands and shorter retention times.

The final elution profiles, generated with 50% methylene chloride : 50% hexane as the sole solvent, are shown in Table 16. Table 16 lists two separate separations as described in section 2.4. The first separation involves the standards and internal standard, while the second separation involves a spiked spruce needle sample.

Table 16 Elution Profile for the Standard/Internal Standard and a Spiked Spruce Needle Sample Using a Single Solvent.

	Fraction Number Standard and Internal Standard Separation							Fraction Number Spiked Spruce Needle Sample Separation					
Pesticide Species	1	2	3	4	5	6		1	2	3	4	5	6
Heptachlor	X								X				
DDT	X							X	X				
d-HCH	X	X						X	X				
Aldrin	X												
DDD		X							X				
DDE	X							X	X				
a-HCH	X	X						X	X				
g-HCH	X	X						X	X				
b-HCH	X	X						X	X				
Endosulfan I				X	X	X					X		
Epoxy Heptachlor		X							X				
Endrin			X					X					
Dieldrin		X	X						X	X			
Chlordanes	X	X							X				
HCB	X							X					
Internal Standard	X							X	X				

The streamlined approach of using only one solvent is adequate. The use of multiple solvents allows for class separation, however this is not necessary for this work. The samples cleaned with the single solvent yielded GC/MS chromatograms with no interfering or co-eluting peaks around the analyte peaks. Therefore silica chromatography for the remainder of the method development and sample analysis was done with the single solvent and the final conditions listed in section 2.4.

From the profile of the spiked sample we see that not all of the analytes are present. However the moderately volatile analytes that may show a cold finger affect (hexachlorobenzene, hexachlorocyclohexanes, and dieldrin) and the internal standard are present. Therefore we used this elution profile for the remainder of the work.

3.3 Lipid Variability within a Single Tree

The GC/MS analysis of analyte variability was not done because of technical problems with the quadrapole. The quadrapole was removed and sent in for replacement. Lipid results were obtained, however. The weights of non-volatile extractables for each of the samples are shown in Table 17.

Table 17 Weights of Nonvolatile Extractables for the Within Tree Variability Investigation.

Sample location and age	Mass extracted (mg) Sample 1	Mass extracted (mg) Sample 2
Top New	356	374
Top Old	335	311
Middle New	436	441
Middle Old	477	439
Bottom New	296	291
Bottom Old	389	372

Table 18 shows an ANOVA table for a two factor analysis of variance with replication for the non-volatile extractable data.

Table 18 ANOVA Table for the Non-Volatile Extractable Data.

Source of Variation	*SS*	*degrees freedom*	*MS*	$F_{calculated}$	$F_{critical}$ 95% Confidence Level
Needle Age	1386.8	1	1386.750	6.202	5.987
Needle Location	31058.2	2	15529.083	69.455	5.143
Interaction	8326.5	2	4163.250	18.621	5.143
Within	1341.5	6	223.583		
Total	42112.9	11			

The analysis of variance indicates that at the 95% confidence level both location and age of needles are correlated with the amount of nonvolatile extractables. The square root of the within group mean square is taken as the within group standard deviation, 15 mg. Table 19 shows the averages for the duplicate measurements, as well as averages for the different locations and ages.

Table 19 Average Values for the Non-Volatile Extractables.

Needle Location	**Needle Age**		**Average** (Location)
	New	**Old**	
Top	365	333	349
Middle	439	458	449
Bottom	294	381	338
Average (Age)	366	391	

Table 19 further shows that the effect of needle age is significant but relatively small. Old needles have approximately 10% more extractables. However the effect of location is large, with needles from the middle of the tree containing levels of nonvolatile extractables approximately 30% higher than needles from the top or bottom of the tree.

The results of the analysis of non-volatile extractables may not reflect within tree variability of pesticides, however these results suggest that all trees should be sampled at approximately the same height and that old and new growth should be homogenized. Therefore, for the subsequent analysis of the tree samples, branches were collected at approximately five feet and the old and new growth needles were combined.

3.4 Extraction Technique

The responses to the first factorial analysis of extraction technique, as described in section 2.5, are in Table 20.

Table 20 Responses to the First Factorial Analysis of Extraction Technique.

Design Number (see tables 6 and 7 section 2.5)	**Mass Before Chromatography** (mg)	**Mass After Chromatography** (mg)
1	275	268
2	377	285
3	202	185
4	431	338
5	173	158
6	591	529
7	103	75
8	597	373

The average difference in treatments between the high and low value for each variable gives the calculated effects, shown in Table 21. For example, the four designs that involved grinding give an average response of 499 mg, while the four designs that were not ground give an average of 188.3 mg. The difference between these gives the calculated effect. A t-test, at the 95% confidence level, determines if the difference in treatments is significant.

Table 21 Calculated Effects for the First Factorial Analysis of Extraction Technique.

Variable	**Before Chromatography**	**After Chromatography**
Grinding	**310.7**	**104.5**
Drying Time (50° c)	-20.8	43.0
Extraction Temperature	44.8	33.0
Extraction Time	50.8	31.5
% Acetone	**145.2**	23.5
Solvent Volume	-11.25	38.0
Agitation	-12.8	36.5
Significant effects are shown in boldface		

The list of effects for the first response indicate grinding and percent acetone are active variables in extraction efficiency. However, after silica chromatography (second response) grinding is the only active variable. This result makes sense. Extracting with 20% acetone will presumable remove more polar compounds from the spruce needles than extracting with pure hexane. However, the majority of these more polar compounds are not eluted from the silica gel. Grinding being an active variable is not surprising, as grinding exposes more of the nonvolatile extractables within the spruce needles.

Listed in Table 22 are the responses to the second factorial analysis of extraction technique, as described in section 2.5. The only analyte observed was hexachlorobenzene. Peak areas were normalized to the volume of sample just prior to GC/MS. Normalizing peak area to volume removes the need to evaporate each sample to exactly 250 µl before GC/MS.

Table 22 Responses to the Second Factorial Analysis of Extraction Technique.

Design Number (see tables 8 and 9 section 2.5)	**HCB Peak Area * Sample Volume (x 10^5)**
1	2.31
2	12.7
3	0
4	9.49
5	4.93
6	9.69
7	6.09
8	9.52
9	1.42
10	5.77
11	7.29
12	9.65
13	9.98
14	10.06
15	6.04
16	11.3

Figure 5 shows the half-normal probability plot for the above responses to the second factorial design. The only active variable for extraction efficiency of HCB is the type of extraction used. Soxhlet extraction is more efficient than soaking.

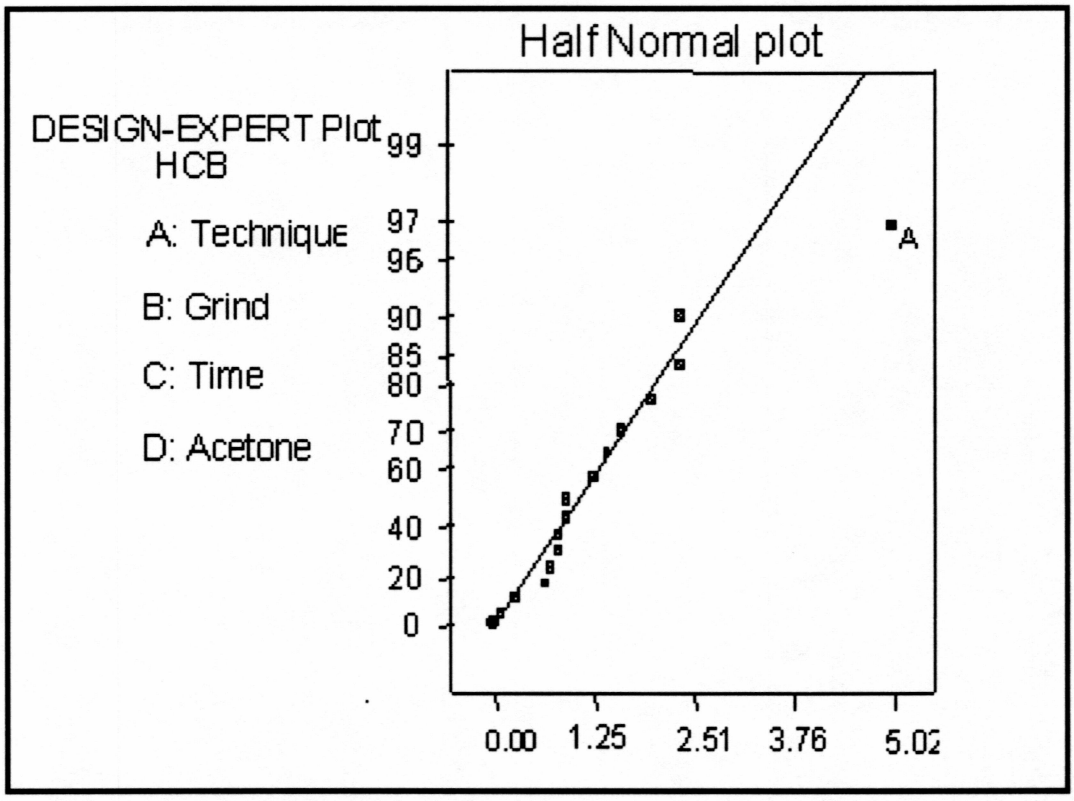

Figure 5 Design Expert Half-Normal Probability Plot for the Second Factorial Analysis of Extraction Technique.

The final extraction approach involved grinding a ten gram sample and extracting with 100 ml hexane in a Soxhlet apparatus for three hours. The time was increased to three hours and the samples were ground because the analysis of the same samples for PAH's done by Tim Howe indicated that grinding and time might be active variables (15).

3.5 Recovery of Analyte and Internal Standard

Standard and internal standard GC/MS peak areas are shown in Table 23 for the investigation of sources of analyte loss during sample work-up, as described in section 2.7.

Table 23 GC/MS Peak Areas for the Source of Analyte Loss Investigation.

Source of Loss =	Control	Rotovap	Evaporate & Dry	Rotovap & Column	All Sources
Samples	Average 3a & 3b	Average 1a & 1b	Average 2a & 2b	Average 4a & 4b	Difference 5a - 5b
Analyte					
Internal Standard	252962	213578	100535	295614	248556
HCB	288392	253123	165296	331556	220944
g-Chlordane	11736	10577	9616	14435	7970
a-Chlordane	3918	3519	3321	4703	2642
a-HCH	162767	148239	97506	201554	288154
b-HCH	138990	130010	116266	168916	149699
g-HCH	132008	123509	96931	155302	139975
d-HCH	72980	65832	62950	93819	98276
Heptachlor	129218	139864	92874	153662	164618
Aldrin	152553	144257	108169	178961	210319
Epoxyheptachlor	134432	170352	102463	150828	145966
Endosulfane I	42960	19088	34679	50831	32585
DDE	281887	251914	239781	342022	200967
Endrin	82404	118814	80819	100677	0
Endrin Ketone	115970	155230	106910	0	27425
Endosulfane Sulfate	18318	13668	17621	13658	0
DDT	129114	153441	123754	184361	116835

The percent recoveries generated by dividing the GC/MS peak areas for the different treatments by the GC/MS peak areas for the control are in Table 24.

Table 24 Percent Recoveries for the Source of Analyte Loss Investigation.

Source of Loss =	Control	Rotovap	Evaporate ± Dry	Rotovap ± Column	All Sources
Samples	3a & 3b	1a & 1b	2a & 2b	4a & 4b	5a - 5b
Analyte					
2,4,5,6-Tetrachloro-m-Xylene	100	84	40	117	98
HCB	100	88	57	115	77
g-Chlordane	100	90	82	123	68
a-Chlordane	100	90	85	120	67
a-HCH	100	91	60	124	177
b-HCH	100	94	84	122	108
g-HCH	100	94	73	118	106
d-HCH	100	90	86	129	135
Heptachlor	100	108	72	119	127
Aldrin	100	95	71	117	138
Epoxyheptachlor	100	127	76	112	109
Endosulfane I	100	44	81	118	76
DDE	100	89	85	121	71
Endrin	100	144	98	122	0
Endrin Ketone	100	134	92	0	24
Endosulfane Sulfate	100	75	96	75	0
DDT	100	119	96	143	90
Average =	**100**	**97**	**78**	**111**	**87**

Evaporating to dryness is the only treatment that shows a significant decrease in percent recovery. Therefore, the sample work-up involved Soxhlet extraction and silica chromatography as described above in sections 3.2 and 3.5. Volume reduction was done by rotary evaporating down to three ml, then evaporation under nitrogen. At no time was the sample allowed to dry out completely.

Figure 6 shows the average values (for all of the 16 analytes) of analyte peak area divided by the internal standard peak area for each of the possible sources of analyte loss.

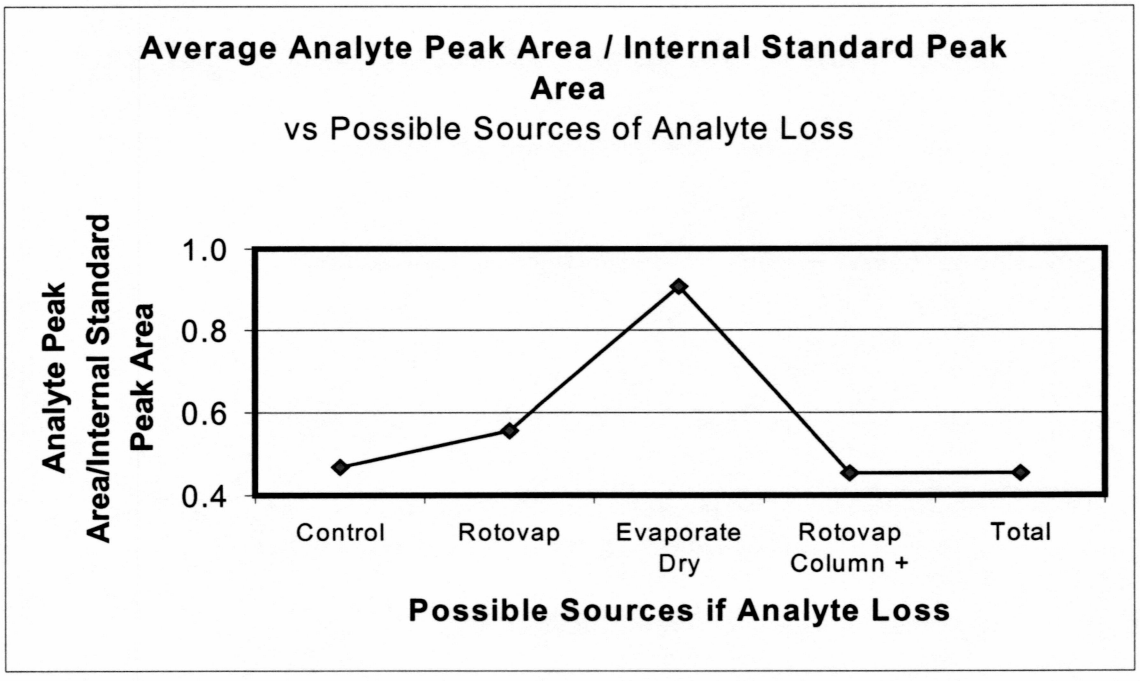

Figure 6 Analyte Peak Areas / Internal Standard Peak Areas for the Source of Analyte Loss Investigation.

Except for samples evaporated to dryness, the ratio of analyte peak area divided by internal standard peak area is relatively constant. This indicates that the internal standard, 2,4,5,6-tetrachloro m-xylene, is somewhat more volatile than the other analytes. Therefore the internal standard will behave like the analytes during sample work-up (i.e. a loss of analyte will be accompanied by an equivalent loss in internal standard) if the sample is not evaporated to dryness. This equivalence allows for correction of analyte loss.

3.6 Quality Control Analysis

The GC/MS results for each auto-sampler sequence log are shown in appendix 2. Each block represent a sequence log consisting of ten needle samples or method blanks and fours runs of the pesticide standard. Hexachlorobenzene was the only analyte found in the tree samples. The corresponding nonvolatile extractable and water content data are listed in appendix 3.

The concentrations of hexachlorobenzene found in the method blanks are listed in Table 25, according to extraction date.

Table 25 Concentration of HCB in Method Blanks.

Extraction Date	[HCB] ppb
11/18/97	13.2
11/19/97	14.7
11/26/97	5.3
12/10/97	0.0
12/12/97	0.0
1/29/98	0.0
2/3/98	0.0
2/11/98	0.0
2/12/98	0.0

Extraction Date	[HCB] ppb
2/13/98	0.0
2/18/98	0.0
2/20/98	0.0
2/26/98	0.0
3/3/98	0.0
3/10/98	0.0
3/11/98	0.0
4/7/98	0.0
4/7/98	0.0

Analyte was found in the method blanks for the first three extraction dates. The source of the analyte in the method blanks was carry over of the spiked samples from the fritted glass filter. Initially a single fritted glass filter was used to filter all samples after soxhlet extraction. Because of time constraints the filter was rinsed with multiple solvents, but not baked, after each use. After the 11/26/98 extraction separate filters were assigned for tree samples, spiked tree samples, and blanks. Subsequent method blanks showed no HCB peaks.

Percent recoveries for HCB in the spiked samples are listed in Table 26.

Table 26 Percent Recoveries of HCB in Spiked Samples.

Highway	Mile	Tree #	Spiked Sample [HCB] ppb	Unspiked Sample [HCB] ppb	Percent Recovery of Spiked Sample
Ak	1230	1	257.4	5.8	101
Ak	1230	2	245.9	5.9	96
Ak	1418	1	254.4	5.4	100
Ak	1418	2	273.8	5.4	107
Dalton	50	1	293.2	11.3	113
Dalton	50	2	278.3	10.9	107
Dalton	193	1	241.8	7.7	94
Dalton	193	2	249.5	5.7	98
Elliot	47	1	155.2	22.0	53
Elliot	47	2	275.6	22.0	101
Parks	107	1	245.6	5.0	96
Parks	107	2	258.3	9.2	100
Parks	230	1	249.4	5.8	97
Parks	230	2	266.3	5.8	104
Rich	16	1	251.6	4.8	99
Rich	16	2	259.5	12.2	99
Rich	166	1	243.2	6.7	95
Rich	166	2	274.4	10.9	105
Rich	309	1	422.9	5.2	167
Rich	309	2	254.7	8.0	99
Seward	42	1	253.6	9.6	98
Seward	42	2	236.6	8.0	91

Nearly all of the spiked samples show essentially complete recovery. Only two of the samples are suspect. Elliot 47-1 shows a low percent recovery, while Richardson 309-1 shows an abnormally high recovery. The above results indicate there is usually little significant analyte loss during sample work-up and analysis.

Calculated values for the percent recovery of the internal standard in the tree samples and the method blanks are listed in appendix 4. Figure 7 represents the resulting distribution of percent recoveries for the internal standard.

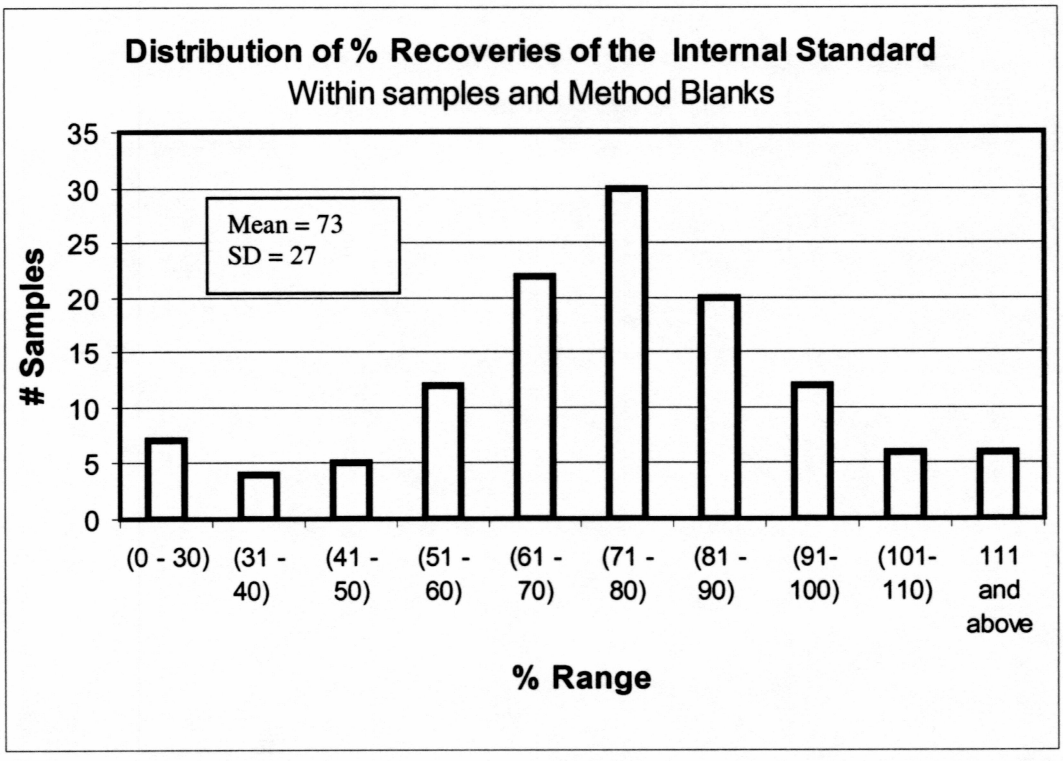

Figure 7 Distribution of Percent Recoveries of the Internal Standard.

Most of the samples and method blanks show recoveries of the internal standard at fifty percent or higher. Tree samples with internal standard percent recoveries less than forty percent were excluded from the final data analysis.

Measurements of HCB concentrations and percent lipid for the tree samples that were replicated are listed in Table 27. Excluded are any replicate pairs that were extracted on a day that gave a non-zero method blank.

Table 27 Values for Replicate Measurements of [HCB] and Percent Lipid.

Group Number	Sample Hyw-Mile-#	Replicate A [HCB] ng/g needles	Replicate B [HCB] ng/g needles	Replicate A % Lipid	Replicate B % Lipid
1	D13-1	1.848	2.773	1.69	1.84
2	D13-2	0.832	0.835	1.40	1.29
3	P147-1	0.511	0.459	0.74	0.78
4	P147-2	0.436	0.615	1.22	1.24
5	P270-1	1.289	1.261	3.50	3.60
6	P270-2	1.885	1.658	4.20	4.31
7	R31-1	0.623	1.173	1.23	1.76
8	R31-2	0.595	0.788	0.99	1.04
9	R339-1	1.070	0.886	2.78	2.80
10	R339-2	0.679	0.788	1.66	1.57
11	S8-1	0.949	0.896	2.53	2.36
12	S8-2	2.094	1.981	1.67	1.94
Pooled Standard Deviation		0.237		0.137	
Relative Pooled SD		21.1		6.84	

Replicate measurements generally show close agreement for both HCB concentrations and lipid content, indicating sound measurements for these qualities.

Percent water measurements were not duplicated as they were calculated from separate individual samples.

3.7 Results of HCB Measurements across Alaska

The chromatogram for the sample at mile 1230 of the Alaska Highway, tree number two, is shown in Figure 8. This chromatogram is typical of the tree samples, showing little or no interfering peaks around the internal standard and HCB.

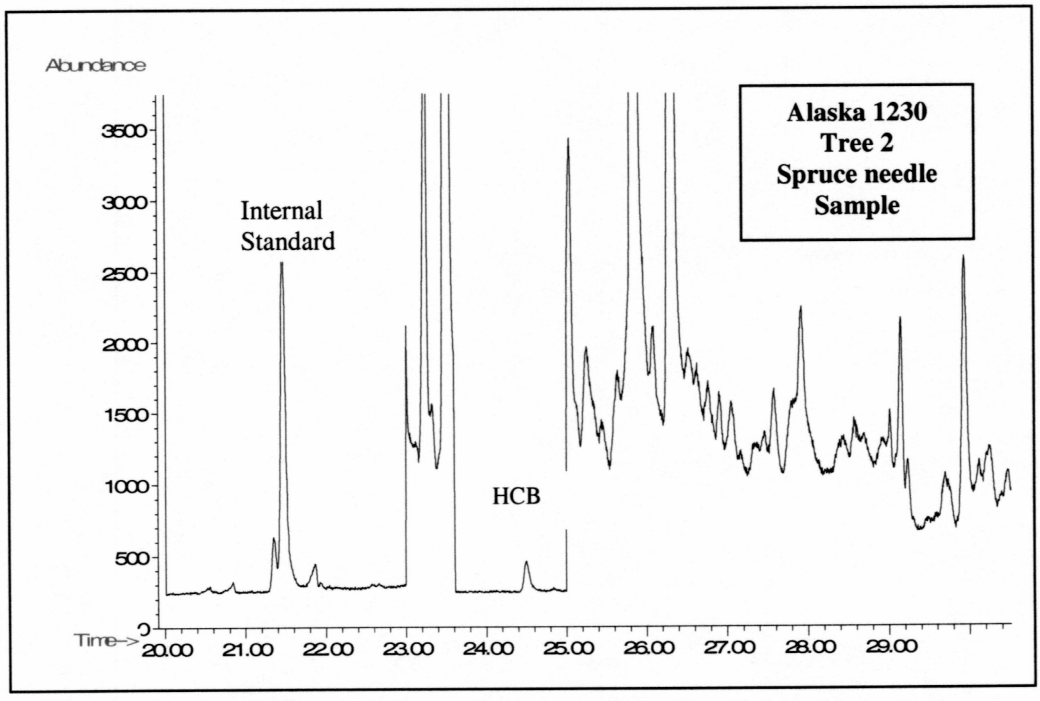

Figure 8 Chromatogram for Alaska Highway Sample 1230 Tree 2.

The results of the HCB analysis of the tree samples are shown in Table 28. Both the concentrations of HCB injected into the GC/MS and nanograms HCB per gram of needle weight are shown.

Table 28 Results of the HCB Analysis of the Spruce Needles.

Highway	Mile	Tree #	Latitude	[HCB]	HCB ng/g needle weight
Alaska	1230	1	62.68	5.75	0.577
Alaska	1230	2	62.68	5.94	0.598
Alaska	1260	1	62.90	7.70	0.769
Alaska	1260	2	62.90		
Alaska *	1301	1a	63.26	15.77	1.578
Alaska	1301	1b	63.26	5.40	0.542
Alaska	1301	2a	63.26		
Alaska	1301	2b	63.26	8.53	0.855
Alaska	1345	1	63.39	5.36	0.536
Alaska	1345	2	63.39	5.01	0.512
Alaska	1384	1	63.70	6.78	0.680
Alaska	1384	2	63.70	5.46	0.546
Alaska	1418	1	63.97	9.61	0.972
Alaska	1418	2	63.97	5.39	0.539
Dalton *	13	1a	65.58	18.46	1.848
Dalton *	13	1b	65.58	27.69	2.773
Dalton	13	2a	65.58	8.31	0.832
Dalton	13	2b	65.58	8.35	0.835
Dalton	50	1	65.85	11.26	1.137
Dalton	50	2	65.85	10.86	1.087
Dalton	87	1	66.27	6.94	0.704
Dalton	87	2	66.27	8.13	0.817
Dalton	122	1	66.67	10.16	1.019
Dalton	122	2	66.67	12.05	1.212
Dalton *	160	1a	67.14	7.79	0.785
Dalton *	160	1b	67.14	15.21	1.529
Dalton *	160	2a	67.14	11.70	1.173
Dalton *	160	2b	67.14	28.15	2.819
Dalton	193	1	67.54	7.70	0.774
Dalton	193	2	67.54	5.73	0.577

Highway	Mile	Tree #	Latitude	[HCB]	HCB ng/g needle weight
Elliot	9	1a	65.09	4.50	0.450
Elliot	9	1b	65.09	7.11	0.714
Elliot	9	2a	65.09	5.81	0.580
Elliot	9	2b	65.09	6.38	0.639
Elliot	47	1	65.36		
Elliot	47	2	65.36	21.81	2.205
Goldstream		1	64.86	10.66	1.067
Goldstream		2	64.86	7.57	0.763
Parks	67	1	62.16	6.79	0.685
Parks	67	2	62.16	4.96	0.501
Parks	107	1	62.69	5.02	0.502
Parks	107	2	62.69	9.20	0.929
Parks	147	1a	63.12	5.10	0.511
Parks	147	1b	63.12	4.57	0.459
Parks	147	2a	63.12	4.34	0.436
Parks	147	2b	63.12	6.10	0.615
Parks	187	1	63.54	5.06	0.507
Parks	187	2	63.54	7.78	0.778
Parks	214	1	63.89	4.63	0.464
Parks	214	2	63.89	7.11	0.716
Parks	230	1	64.10	5.83	0.590
Parks	230	2	64.10	5.81	0.593
Parks	270	1a	64.59	12.72	1.289
Parks	270	1b	64.59	12.57	1.261
Parks	270	2a	64.59	18.82	1.885
Parks	270	2b	64.59	16.52	1.658
Richardson	4	1	61.10	7.54	0.753
Richardson	4	2	61.10	6.98	0.697
Richardson *	16	1	61.09	4.67	0.471
Richardson *	16	2	61.09	12.18	1.218
Richardson	31	1a	61.18	6.21	0.623
Richardson *	31	1b	61.18	11.70	1.173
Richardson	31	2a	61.18	5.91	0.595
Richardson	31	2b	61.18	7.87	0.788
Richardson	72	1	61.60	9.24	0.925
Richardson	72	2	61.60	6.87	0.691
Richardson	109	1	62.05	0.00	
Richardson	109	2	62.05	6.52	0.651

Highway	Mile	Tree #	Latitude	[HCB]	HCB ng/g needle weight
Richardson	166	1	62.82	6.62	0.673
Richardson	166	2	62.82	10.91	1.091
Richardson	207	2a	63.33	5.17	0.521
Richardson	207	2b	63.33	5.84	0.586
Richardson	277	1	64.23	7.78	0.788
Richardson	277	2	64.23	6.39	0.643
Richardson	309	1	64.37	5.19	0.521
Richardson	309	2	64.37	8.03	0.811
Richardson	339	1a	64.72	10.63	1.070
Richardson	339	1b	64.72	8.82	0.886
Richardson	339	2a	64.72	6.76	0.679
Richardson	339	2b	64.72	7.86	0.788
Seward	8	1a	60.14	9.43	0.949
Seward	8	1b	60.14	8.88	0.896
Seward *	8	2a	60.14	20.96	2.094
Seward *	8	2b	60.14	19.70	1.981
Seward	42	1	60.65	9.56	0.962
Seward	42	2	60.65	7.98	0.802
Seward	75	2a	60.83	6.31	0.647

* Samples Excluded from Final Analysis (Criteria Described in section 2.8)

The chromatograms, all spreadsheets, and a copy of this thesis are available on CD-ROM at The UAF Chemistry Department.

3.8 Interpretation of HCB Measurements across Alaska

The primary concern of this work is to look for latitude dependent concentration gradients, as an indication of a cold finger effect. A plot of HCB versus latitude is shown in Figure 9.

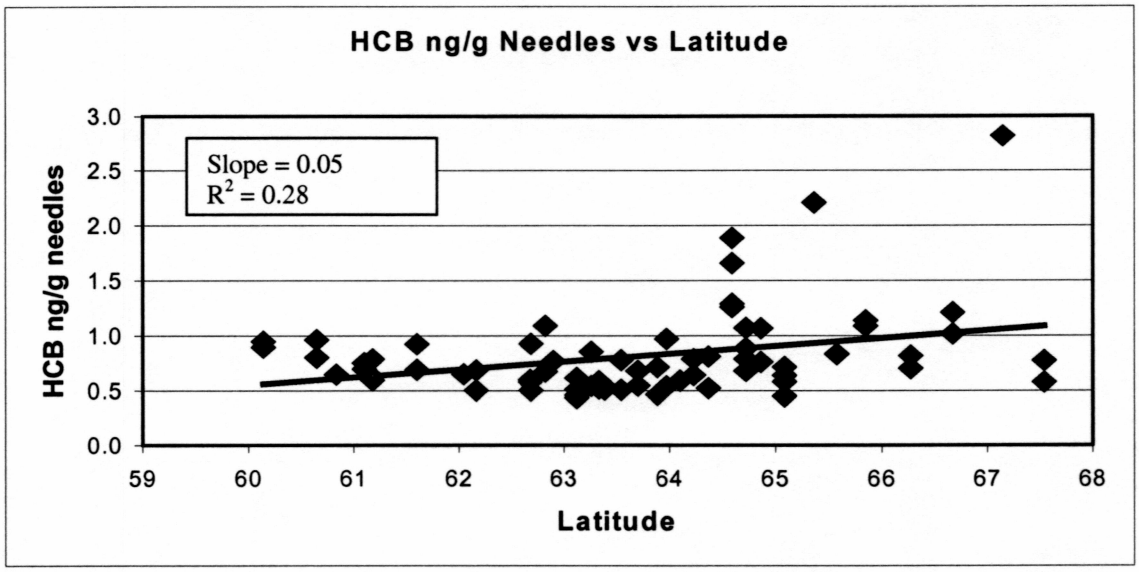

Figure 9 HCB ng/g Dry Weight vs. Latitude for the Spruce Needle Samples.

The above univariate regression plot does not strongly support a latitude dependent concentration gradient. The scattered distribution of the data results in a low correlation between HCB and latitude. The univariate regression fails, as HCB may be dependent on any of the following variables: latitude, elevation, terrain, tree species, average temperature, needle lipid content, radial distance to the closest city, and average precipitation. In addition, these X variables may correlate with each other. Any analysis of HCB must be multivariate, taking into consideration all the X variables and how they correlate with HCB and each other.

For this work principle component regression was employed, as described in section 2.8. Projection of least squares with one response (PLS1) was used, as this mode of principle component regression is compatible with data sets that contain correlation between the X variables.

Validation and calibration residual variances are shown in Figure 10 for principle components one through six for the PLS1 model of the tree samples.

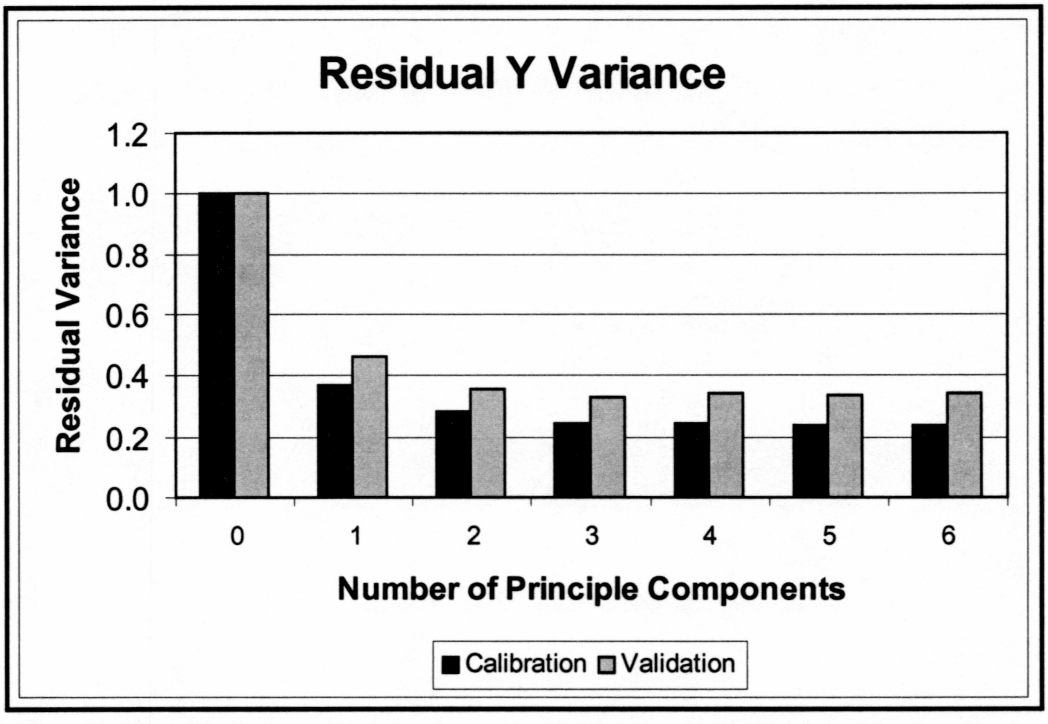

Figure 10 Residual Variance for the First Multivariate Analysis.

The calibration and validation variance values suggest an optimum of one principle component for the multivariate model describing HCB. Using additional principle components gives only slight decreases in residual variance, and may result in overfitting, as higher principle components are most likely modeling noise in the data set rather than structure.

Correlation among the variables and how the variables define the principle components are represented by the X-Loading weights and Y-loadings plot. This plot is shown in Figure 11 for principle components one and two, with the X axis representing PC1 and the Y axis representing PC2.

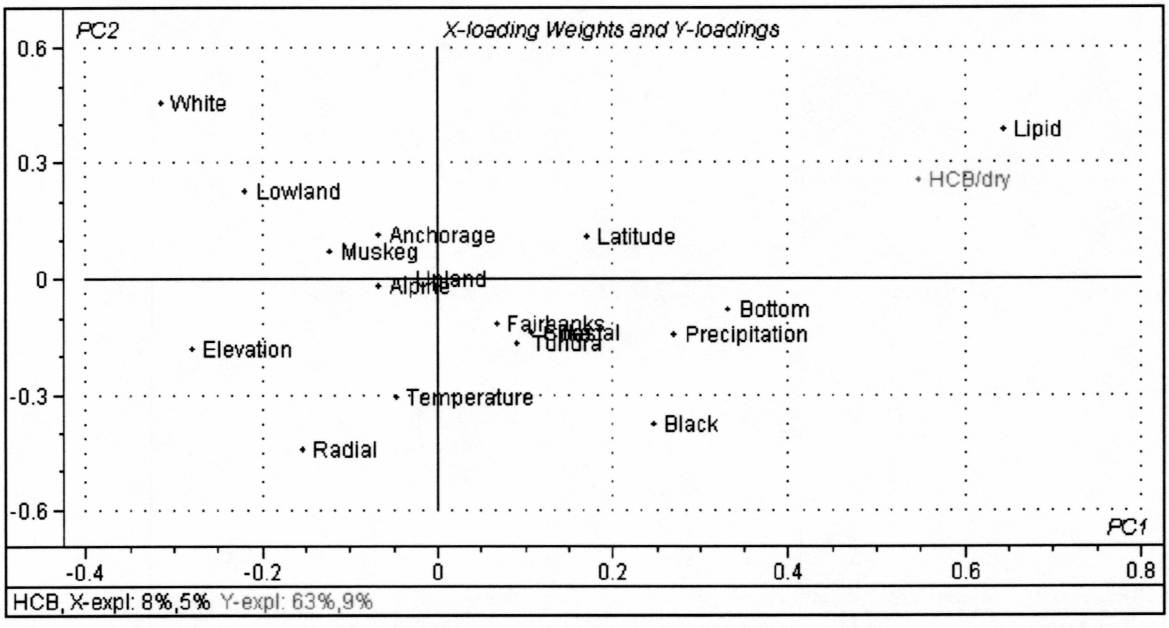

Figure 11 X-Loading Weights and Y-Loadings for the First Model.

The plot must be interpreted carefully as it represents correlation among X variables, correlation between the X variables and the response, and how the variables describe the principle components. Variables that lie close to one another may be positively correlated, while variables that lie far apart may be negatively correlated. Also, variables with large values along a principle component may be large contributors to the principle component.

The values in the lower left corner of the X-Loading weights and Y-loadings plot describe how much of the explained variance corresponds to each principle component. For this model PC1 encompasses 8% of the variation in the X variables and explains 63% of the variation in the HCB concentrations. PC2 encompasses an additional 5% of the variation in the X variables but only explains an additional 9% of the variation in the HCB concentrations. PC2 does not describe much of the variation in the HCB data, therefore the model is based on one principle component and interpretation of relationships in Figure 11 is done along the x axis only.

PC1 describes the variation in HCB as positively correlated primarily with lipid content. Figure 12 shows this correlation clearly.

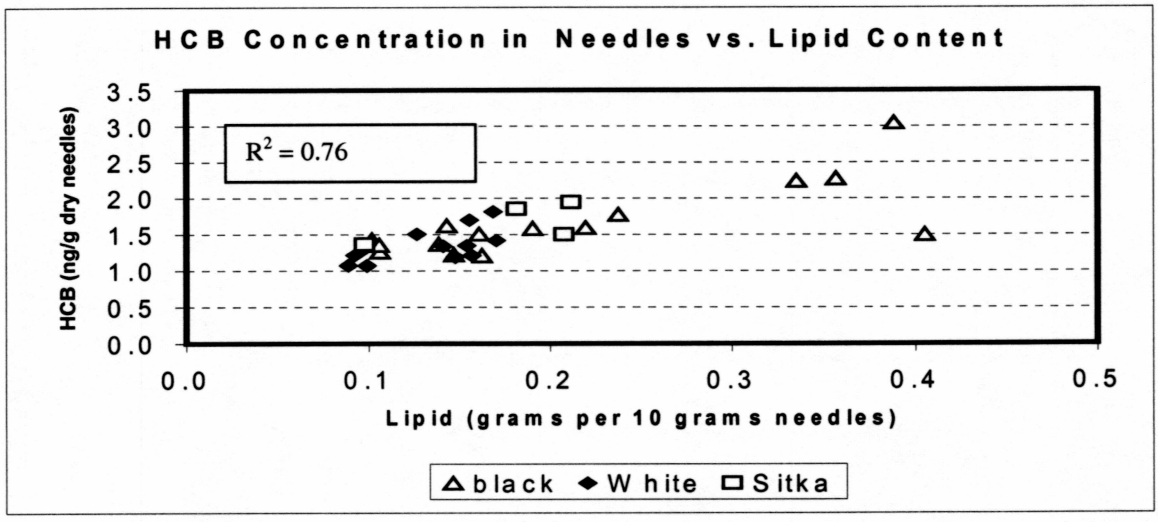

Figure 12 HCB Measurements versus Needle Lipid Content.

Black spruce show the highest HCB and lipid measurements, while white spruce shows the lowest. This is in agreement with the distribution of the tree types along PC1.

Figure 11 describes a model, based on one principle component, that relates the structure in the HCB concentrations solely to lipid content. However, there is a deficiency with the model. Figure 13 shows a three-dimensional scatter plot of HCB levels, lipid content, and latitude.

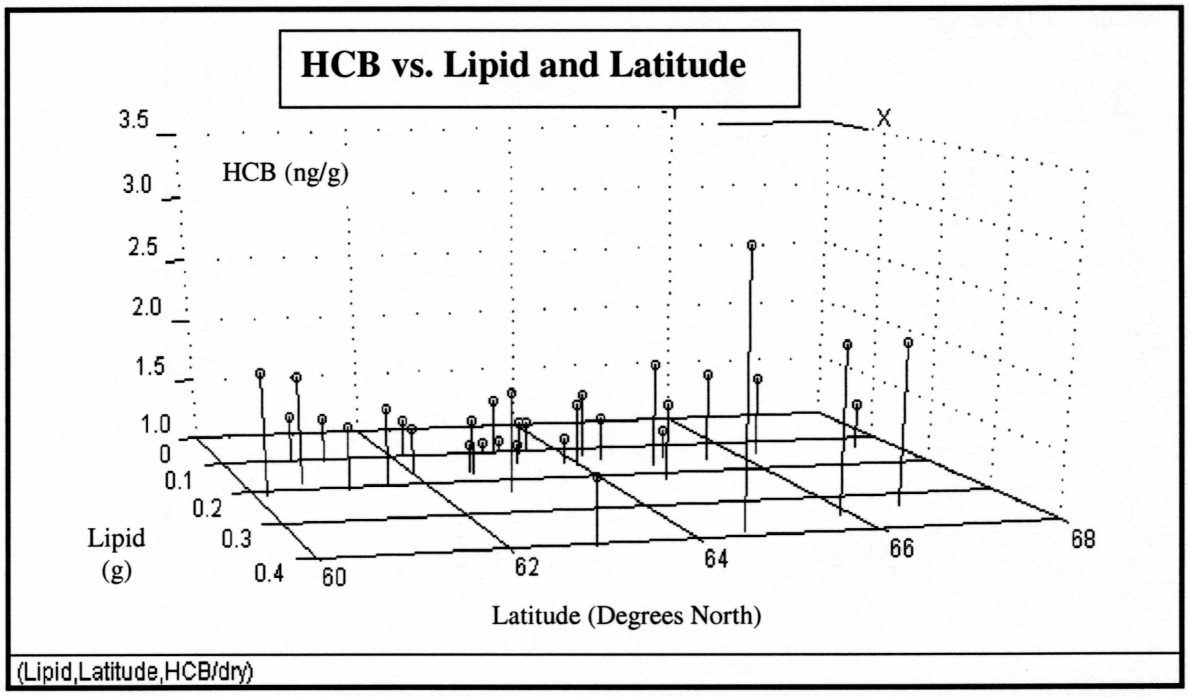

Figure 13 HCB vs. Lipid and Latitude.

There appears to be a nonlinear relationship between HCB concentrations and latitude. The Unscrambler looks for structure in the response that can be attributed to linear relationships between the response and the X variables. However, the relationship between HCB levels and latitude is not linear. Sample sites south of 63^0 indicate a possible negative correlation between HCB levels and latitude, while the remaining sample sites indicate a possible positive correlation. These two opposite trends give a net correlation indistinguishable from zero.

One solution is to make a distinction between the samples north and south of the minimum HCB concentration, which appears to be at approximately 63^0. For the second model latitude is replaced with two variables, north of 63^0 and south of 63^0. Each sample contains a value for only one of these two new variables, while the other variable is recorded as missing. Two examples are shown below.

Sample	Latitude	North of 63^0	South of 63^0
Dalton mile 13	65.58^0	2.58	Missing
Seward mile 75	60.83^0	Missing	0.83

The second model required the removal of two sample site, P 65.59 and R 61.18, as they were identified as strong outliers. The X-loading Weights and Y-loadings for PC1 and PC2 of the second model are shown in Figure 14.

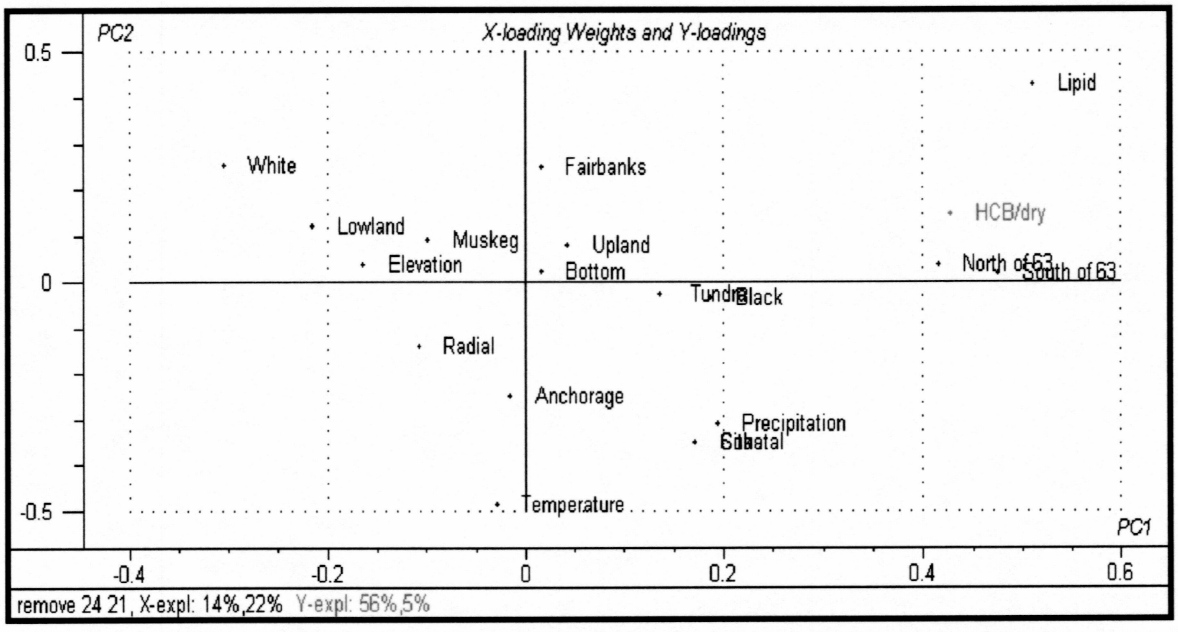

Figure 14 X-Loading Weights and Y-Loadings for the Second Model.

This second model is similar to the first model. PC1 describes 56% of the variation in the HCB levels, while PC2 only describes 5%. Again the model should be based on one principle component. This model also identifies lipid as the predominant variable describing HCB levels, and that the distribution of species shown in Figure 12 still applies.

The primary difference between the first model and this new model involves the additional X variables. Both new X variables, north of 63^0 and south of 63^0, have significant X-loading weights along PC1. The significant X-loading weights indicate the presence of two significant latitude trends. HCB concentrations show a negative correlation with latitude from the coast to approximately 63^0, and a positive correlation from approximately 63^0 northward. This positive correlation between latitude and HCB concentrations is consistent with the cold finger effect.

Shown in Figure 15 are the measured vs. predicted values based on the second model. Two sets of measured versus predicted values are shown. The black values represent the calibration set, while the red values represent the validation set.

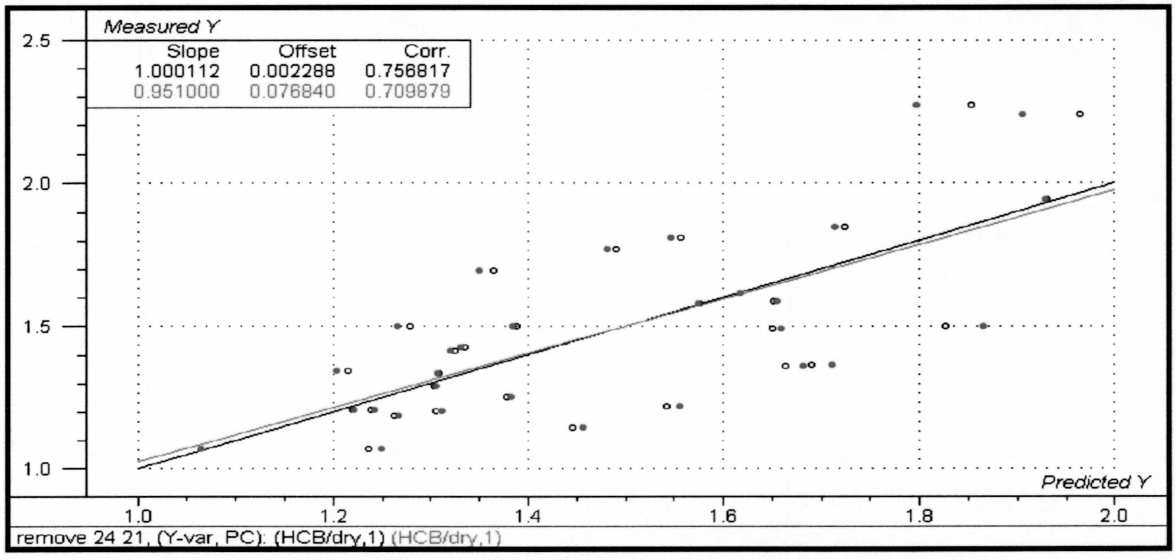

Figure 15 Measured vs. Predicted HCB Levels for the Second Model.

The calibration predicted versus measured set compares HCB values predicted by the one PC model to measured HCB values. This gives a measure of how well the model describes the data.

The validation predicted versus measured set compares HCB values predicted by a validation technique to measured HCB values. This comparison confirms the quality of the model and may offer a measure of how well the model can predict future values. Leverage correction is the validation technique used here. Leverage correction calculates the validation residual (the difference between the line Y=X, in Figure 15, and the predicted value) as the calibration residual divided by (1 - sample leverage). This approach is the least conservative validation approach and may give overly optimistic results when the goal of the model is to predict future values. However, for this study future prediction is not a goal and the leverage correction technique is adequate for confirming the quality of the model. If any of the sample sites are skewing the model then they will show validation residuals that are significantly larger than calibration residuals, in Figure 15.

Regression lines and corresponding slope, intercept, and correlation values are also shown in Figure 15. A slope of one, an intercept of zero, and a correlation of one would indicate a perfect fit for the model. The values for the calibration, and more importantly the validation, for these three parameters suggest a quality fit for the model. Residuals are random and without structure, and validation values are not significantly different from calibration values.

A quantitative measure of the quality of this fit is given by the root mean square error (RMSE). The RMSE is the average residual from the predicted versus measured graph, in original units. The second model gives a RMSE of 0.22. Dividing the RMSE by the average HCB concentration gives an average error of 13% for the calibration and 15% for the validation. This relative error for the model is quite low in light of the 20% variability seen in the duplicate measurements.

The one principle component model therefore describes the structured variation in the tree sample HCB levels, with HCB levels, lipid content, north of 63^0, and south of 63^0 dominating PC1. The relatively high calibration and validation correlation values indicate that the simple one principle component model fits the data well. The RMSE calculates an average difference between calculated HCB levels and measures levels of only 15%. The resulting model greatly reduces the complexity of the data set and gives a big picture of how the X variables correlate to one another and to HCB.

Chapter 4
Conclusion

Many samples collected for environmental studies are processed using traditional procedures, applied in a "cook book" type approach. This type of may give acceptable results, however resources may be wasted and opportunities for observing significant effects may be lost. During the course of this work, sample preparation and data analysis techniques were investigated in order to find methods that are efficient in terms of time, materials, and data evaluation.

The investigation of sample extractions suggested a two or three hour soxhlet extraction as the best approach. Soxhlet proved to be more efficient than soaking at extracting HCB. However, a 22 hour extraction offered no benefit over 3 hour extraction. Grinding the sample and adding acetone to the hexane showed no increase in extraction efficiency.

Sample cleanup in preparation for GC/MS analysis was also simplified. Traditional techniques may include multiple chromatography techniques, frequently with multiple solvent elution profiles. However, a simple column chromatography approach using silica and a single solvent elution profile gave good results. The GC/MS analysis, using this simple cleanup approach and selected ion monitoring, yielded chromatograms with no interfering peaks.

The investigation of possible sources of analyte loss showed rotovaping to dryness as the only significant factor. All other steps involved in sample preparation showed no significant analyte loss. Subsequently samples for this study were not evaporated to dryness during sample work-up.

The multivariate analysis of the HCB data resulted in a statistically sound and relatively simple model based upon one principle component. The model identifies lipid content and adjusted latitude as the major variables defining the structured variation in the HCB levels. For the calibration and validation this model yielded correlation values of 0.86 and 0.78 respectively. Root mean square error values show that the one principle component model fits the HCB data with a relative standard deviation of 15%.

The observation that analyte concentrations in plant samples depend on lipid content has been made before. Hites has suggested that the use of vegetation samples as passive air samplers requires normalizing analyte concentration to lipid content (12). Normalizing to lipid simply takes into account the quantity of lipid phase in the sample available for phase transfer of analyte from the atmosphere. However, for the current work normalizing HCB to the needle lipid content was not necessary, as multivariate analysis is capable of calculating if and how each of the X variables contributes to the variations in HCB measurements.

The most interesting and important aspect of the multivariate analysis is the relationship between HCB and latitude. The multivariate analysis clearly identifies a negative correlation between HCB levels and latitude from the southern coast to approximately 63^0, and a positive correlation from approximately 63^0 northward.

This positive correlation between latitude and HCB levels is consistent with the cold finger theory. Increasing latitude is accompanied by decreasing average temperatures with the effect being an increased partitioning of HCB from the air to the lipid of the spruce needle samples, as the vapor pressure of HCB decreases.

The samples between 60^0 and 63^0 show relatively high HCB levels, and a latitude trend opposite that predicted by the cold finger theory. Interestingly these sample range from the coast to the southern side of the Alaska Range. Some feature of this region appears to make it distinctly different from interior Alaska.

One possible interpretation of this study is that the cold finger effect is real, and that a compounds thermodynamic properties will influence the nature of its global long range atmospheric transport and partitioning. However, the cold finger theory has limitations, and its influence on spatial concentration trends may be masked by relatively short range influences.

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Appendix 1 Sample Site Descriptions

highway	mile	tree #	species	Latitude degree	minutes	Longitude degree	minutes	Elevation	ecosystem
Rich	4	1	sitka	61	6	146	12.35	200	c
Rich	16	1	sitka	61	5.57	145	51.91	400	c
Rich	31	1	black	61	10.72	145	38.06	2000	a
Rich	72	1	black	61	36	145	12.36	1750	u
Rich	109	1	white	62	4.71	145	22.06	1420	l
Rich	166	1	black	62	49.43	145	29.11	2800	u
Rich	207	1		63	19.93	145	42.36	2500	mt
Rich	239	1		63	45	145	47.66	2500	u
Rich	277	1	white	64	13.72	146	0	1000	mb
Rich	309	1	white	64	22.29	146	50.3	800	mb
Rich	339	1	black	64	43.29	147	13.24	500	b
Dalton	13	1	white	65	34.71	148	59.1	1100	mb
Dalton	50	1	black	65	51	149	40.59	1100	u
Dalton	87	1	black	66	16.29	150	17.65	1500	u
Dalton	122	1	black	66	40.29	150	37.93	850	mt
Dalton	160	1	black	67	8.57	150	17.65	900	u
Dalton	193	1	black	67	32.14	149	46.77	1500	u
Elliot	9	1	black	65	5.14	147	34.97	600	mb
Elliot	47	1	white	65	21.86	148	14.12	800	u
Parks	67	1	white	62	9.86	150	7.06	400	l
Parks	107	1	white	62	41.14	150	13.24	800	u
Parks	147	1	white	63	7.29	149	26.48	1800	u
Parks	187	1	black	63	32.57	148	46.77	2000	u
Parks	214	1	white	63	53.14	149	1.77	1400	u
Parks	230	1	white	64	6	149	12.36	1000	l

Appendix 1 Sample Site Descriptions

Parks	270	1	black	64	35.57	149	6.18	300	b
Seward	8	1	sitka	60	8.57	149	22.95	200	c
Seward	45	1	sitka	60	38.79	149	30.45	1400	c
Seward	75	1	sitka	60	49.93	148	59.13	0	c
AK	1230	1	black	62	40.72	141	5.3	2100	mb
AK	1260	1	black	62	54	141	31.77	1900	u
AK	1301	1	black	63	15.43	142	24.71	1800	b
AK	1345	1	white	63	23.15	143	47.64	1500	l
AK	1384	1	white	63	42	144	37.06	1300	u
AK	1418	1	black	63	58.29	145	30	1200	l
Goldstream		1	white	64	51.86	147	52.94	482	u

KEY for ecosystem

c	coastal western hemlock-sitka spruce forest
mt	moist tundra
u	upland spruce hardwood forest
l	lowland spruce hardwood forest
mb	muskeg-bog
a	alpine tundra
b	bottomland spruce forest

Appendix 2 GC/MS Results from the Analysis of the Tree Samples

Each Block represents a Sequence Log for the Auto-Sampler

Extraction Date	Highway	Mile	Tree #	Volume Sample ml	Peak Areas Internal Standard	Peak Areas HCB	HCB / Int Std	[HCB] ppb		Standard Average HCB/INT STD
	stda1				12416	24489	1.972			1.952
2/20/98	Blank 2-20			0.400	7880	0	0.000	0.00		
2/13/98	Rich	339	1a	0.253	12239	1016	0.083	10.63		
2/11/98	Parks	67	2	0.245	13459	521	0.039	4.96		
2/13/98	Dalton	193	2-spike	0.272	11355	22127	1.949	249.54		
2/13/98	Ak	1260	1	0.370	0	670		0.00		
	stda2				15235	30162	1.980			
	stdb1				14375	27889	1.940			
2/13/98	Dalton	193	1-spike	0.222	13788	26037	1.888	241.82		
2/12/98	Ak	1418	2-spike	0.390	6168	13190	2.138	273.84		
2/3/98	Ak	1418	1	0.230	11767	882	0.075	9.60		
12/12/97	Blank 12-12			0.335	7017	0	0.000	0.00		
2/12/98	Parks	147	2a	0.291	9784	332	0.034	4.35		
	stdb2				14521	27834	1.917			
	stda1				29147	53992	1.852			1.827
4/7/98	blank 4/7			0.3370	0	0	0.000	0.00		
4/7/98	Elliot	47	2	0.2800	7418	1194	0.161	22.02		
4/7/98	Parks	230	1-spike	0.4500	10050	18320	1.823	249.40		
4/7/98	Goldstream		1	0.3210	8454	665	0.079	10.76		
	stda2				27387	50076	1.828			
	stdb1				29738	53795	1.809			
4/7/98	Goldstream		2	0.4700	11022	616	0.056	7.65		
4/7/98	Elliot	47	2-spike	0.2900	10876	21909	2.014	275.60		
4/7/98	Rich	166	1-spike	0.4490	11104	19736	1.777	243.17		
4/7/98	Rich	166	1	0.6450	6486	317	0.049	6.69		
	stdb2				28891	52563	1.819			

Appendix 2 GC/MS Results from the Analysis of the Tree Samples

Extraction Date	Highway	Mile	Tree #	Volume Sample ml	Peak Areas Internal Standard	HCB	HCB / Int Std	[HCB] ppb		Standard Average HCB/INT STD
	stda1				22490	39734	1.767			1.787
3/11/98	Rich	4	2	0.2500	15882	792	0.050	6.98		
3/11/98	Parks	107	1-spike	0.4500	10310	18100	1.756	245.56		
3/11/98	Rich	166	2	0.2600	13865	1081	0.078	10.91		
3/11/98	Dalton	122	1	0.3750	8044	584	0.073	10.16		
3/11/98	Ak	1345	1	0.2550	14033	537	0.038	5.35		
	stda2				22035	39177	1.778			
	stdb1				24096	43448	1.803			
3/10/98	Dalton	50	2	0.2650	11897	923	0.078	10.85		
4/7/98	Blank 4/7			0.2880	14341	0	0.000	0.00		
4/7/98	Parks	270	2a	0.3300	6142	826	0.134	18.81		
4/7/98	Parks	230	1	0.2940	13640	569	0.042	5.83		
4/7/98	Parks	270	2b	0.2560	7500	886	0.118	16.52		
	stdb2				18921	34084	1.801			
	stda1				13264	27088	2.042			1.966
2/18/98	Seward	42	2	0.3850	7866	494	0.063	7.98		
2/20/98	Rich	309	2	0.3620	8692	549	0.063	8.03		
2/12/98	Blank 2/12			0.3120	8067	0	0.000	0.00		
2/18/98	Blank 2/12			0.4250	840	0	0.000	0.00		
2/12/98	Ak	1418	2	0.2550	10537	447	0.042	5.39		
	stda2				12742	25012	1.963			
	stdb1				13276	26375	1.987			
2/20/98	Seward	42	1	0.3800	6274	472	0.075	9.56		
2/18/98	Seward	42	2-spike	0.2400	9151	17028	1.861	236.58		
2/20/98	Rich	277	1	0.4000	6245	382	0.061	7.78		
2/12/98	Dalton	193	1	Tim Lost I	10718	649	0.061	7.70		
2/11/98	Blank 2/11			0.3520	6118	0	0.000	0.00		
	stdb2				12175	22815	1.874			

Appendix 2 GC/MS Results from the Analysis of the Tree Samples

Extraction Date	Highway	Mile	Tree #	Volume Sample ml	Peak Areas Internal Standard	HCB	HCB / Int Std	[HCB] ppb		Standard Average HCB/INT STD
	stda1				12055	22332	1.853			1.975
2/18/98	Rich	16	1-spike	0.3850	6083	12088	1.987	251.60		
2/18/98	Rich	16	1	0.3790	11408	421	0.037	4.67		
11/26/97	Elliot	9	1b	0.3900	5425	305	0.056	7.12		
12/12/97	Rich	309	1	0.3480	6605	271	0.041	5.19		
12/10/97	Dalton	13	2b	0.2800	7440	491	0.066	8.36		
	stda2				10235	21118	2.063			
	stdb1				10617	21307	2.007			
2/11/98	Parks	147	1a	0.3450	6730	271	0.040	5.10		
11/3/97	Seward	8	2a	0.4100	4764	789	0.166	20.97		
12/10/97	Blank 12-10				7799	0	0.000	0.00		
11/18/97	Ak	1301	1a	0.2600	6551	816	0.125	15.77		
11/26/97	Blank 11-26				6126	255	0.042	5.27		
	stdb2				10713	21170	1.976			

	stda1				16265	31028	1.908			1.908
11/3/97	Seward	8	2b	0.3200	10875	1634	0.150	19.69		
12/10/97	Rich	339	2a	0.2500	11675	603	0.052	6.77		
11/19/97	Ak	1301	2a	0.3200	1183	655	0.554	72.56		
12/12/97	Rich	31	1a	0.2590	10258	486	0.047	6.21		
11/19/97	Blank 11-19			0.1550	18319	2055	0.112	14.70		
	stda2				13925	26794	1.924			
	stdb1				10470	20291	1.938			
2/13/98	Blank 2-13			0.2520	13768	0	0.000	0.00		
12/10/97	Dalton	13	2a	0.3550	8501	539	0.063	8.31		
11/18/97	Dalton	160	2a	0.1800	12078	1078	0.089	11.70		
12/12/97	Rich	31	1b	0.3850	6576	405	0.062	8.07		
11/19/97	Ak	1301	2b	0.2800	8636	562	0.065	8.53		
	stdb2				13343	24824	1.860			

Appendix 2 GC/MS Results from the Analysis of the Tree Samples

Extraction Date	Highway	Mile	Tree #	Volume Sample ml	Peak Areas Internal Standard	Peak Areas HCB	HCB / Int Std	[HCB] ppb		Standard Average HCB/INT STD
	stda1				31529	63119	2.002			1.965
3/3/98	Ak	1230	1-spike	0.3840	15285	30913	2.022	257.36		
3/3/98	Dalton	50	1	0.3600	12504	1106	0.088	11.26		
3/3/98	Ak	1230	1	0.3420	12978	586	0.045	5.75		
3/3/98	Blank 3-3			0.2500	18246	0	0.000	0.00		
3/3/98	Dalton	122	2	0.1620	17719	1678	0.095	12.05		
	stda2				22411	44345	1.979			
	stdb1				24413	46615	1.909			
2/26/98	Ak	1384	2	0.2700	17120	735	0.043	5.46		
2/26/98	Parks	230	2	0.2720	15129	692	0.046	5.82		
2/26/98	Parks	270	1a	0.2550	11163	1116	0.100	12.72		
3/3/98	Seward	8	1b	0.3100	11532	805	0.070	8.88		
3/3/98	Dalton	87	2	0.1620	20061	1281	0.064	8.13		
	stdb2				21935	43173	1.968			
	stda1				28421	55308	1.946			1.877
3/3/98	Seward	8	1a	0.2540	20531	1453	0.071	9.43		
2/26/98	Rich	277	2	0.3620	13722	658	0.048	6.39		
2/26/98	Ak	1384	1	0.3240	14949	761	0.051	6.78		
3/3/98	Dalton	50	1-spike	0.2920	13032	28687	2.201	293.21		
3/3/98	Ak	1230	2-spike	0.2850	16493	30452	1.846	245.93		
	stda2				27419	50931	1.858			
	stdb1				38663	70974	1.836			
3/11/98	Parks	187	2	0.3350	23718	1385	0.058	7.78		
3/3/98	Ak	1230	2	0.2880	18893	843	0.045	5.94		
2/26/98	Parks	214	2	0.2860	17934	957	0.053	7.11		
3/3/98	Rich	72	2	0.2440	20329	1048	0.052	6.87		
3/10/98	Rich	4	1	0.3750	11858	671	0.057	7.54		
	stdb2				37518	70091	1.868			

Appendix 2 GC/MS Results from the Analysis of the Tree Samples

Extraction Date	Highway	Mile	Tree #	Volume Sample ml	Peak Areas Internal Standard	HCB	HCB / Int Std	[HCB] ppb		Standard Average HCB/INT STD
	stda1				28956	54553	1.884			1.872
2/26/98	Rich	16	2	0.2100	27547	2513	0.091	12.18		
1/29/98	Rich	31	2b	0.2800	23220	1358	0.058	7.81		
2/13/98	Dalton	193	2	0.2380	21265	912	0.043	5.73		
2/12/98	Parks	147	2b	0.3040	14478	661	0.046	6.10		
2/20/98	Rich	309	2-spike	0.2550	18008	34342	1.907	254.73		
	stda2				24492	44364	1.811			
	stdb1				26857	50409	1.877			
2/26/98	Parks	214	1	0.2950	14073	489	0.035	4.64		
2/26/98	Parks	230	2-spike	0.3880	11846	23618	1.994	266.30		
2/26/98	Parks	270	1b	0.3920	13044	1227	0.094	12.56		
2/26/98	Rich	16	2-spike	0.3680	11404	22157	1.943	259.51		
2/26/98	Blank 2/26			0.3500	10332	0	0.000	0.00		
	stdb2				24387	46692	1.915			

	std 1a				26669	23837	0.894			0.922
	std 1b				15408	23837	1.547		241.52	1.601
11/26/97	Rich	207	2b	0.3100	16155	605	0.037	10.15	5.85	
11/19/97	Dalton	160	1b	0.3000	9299	906	0.097	26.41	15.21	
11/3/97	Rich	109	1	0.3500	9343	0	0.000	0.00	0.00	
11/26/98	Rich	207	2a	0.2650	24860	822	0.033	8.96	5.16	
11/3/97	Dalton	13	1a	0.2950	8816	1042	0.118	32.04	18.45	
	std 2a				18554	16736	0.902			
	std 2b				10755	16736	1.556			
11/3/97	Dalton	13	1b	0.2250	8577	1521	0.177	48.07	27.68	
11/18/97	Blank 11/18			0.2800	10590	515	0.049	13.18	7.59	
11/26/97	Elliot	9	1a	0.4150	8983	259	0.029	7.82	4.50	
11/19/97	Dalton	160	1a	0.3300	8910	445	0.050	13.54	7.80	
11/3/97	Ak	1260	2	0.4100	0	0	0.000	0.00	0.00	
	std 3a				14972	14536	0.971			
	std3b				8546	14536	1.701			

Appendix 2 GC/MS Results from the Analysis of the Tree Samples

Extraction Date	Highway	Mile	Tree #	Volume Sample ml	Peak Areas Internal Standard	HCB	HCB / Int Std	[HCB] ppb		Standard Average HCB/INT STD
	stda1				23147	41560	1.795			1.800
2/3/98	Parks	67	1	0.3440	15750	770	0.049	6.79		
2/3/98	Ak	1418	1-spike	0.3440	15282	27998	1.832	254.39		
2/3/98	Blank 2/3			0.4600	9636	0	0.000	0.00		
2/11/98	Elliot	9	2b	0.2620	15154	696	0.046	6.38		
2/3/98	Elliot	47	1-spike	0.3420	21170	23667	1.118	155.23		
	stda2				21263	38245	1.799			
	stdb1				21592	39109	1.811			
2/3/98	Elliot	47	1	0.2100	0	1461				
2/11/98	Elliot	9	2a	0.3400	12619	528	0.042	5.81		
2/11/98	Parks	147	1b	0.3680	10897	358	0.033	4.56		
2/20/98	Seward	42	1-spike	0.3200	10605	19370	1.827	253.62		
2/18/98	Dalton	87	1	0.3380	11394	570	0.050	6.95		
	stdb2				19726	35439	1.797			

	stda1				32180	63696	1.979			1.923
3/10/98	Blank 3/10			0.2050	29110	0	0.000	0.00		
3/11/98	Parks	107	1	0.3100	20438	788	0.039	5.01		
3/11/98	Blank 3/11			0.3650	12826	0	0.000	0.00		
3/10/98	Parks	107	2	0.3400	12842	909	0.071	9.20		
3/10/98	Rich	72	1	0.3750	13318	947	0.071	9.24		
	stda2				29430	56956	1.935			
	stdb1				32897	62209	1.891			
3/11/98	Ak	1345	2	0.3750	17396	670	0.039	5.01		
3/10/98	Parks	107	2-spike	0.3300	12733	25300	1.987	258.28		
3/11/98	Parks	187	1	0.3500	16213	630	0.039	5.05		
3/10/98	Dalton	50	2-spike	0.2000	14295	30606	2.141	278.30		
3/11/98	Rich	166	2-spike	0.2750	14263	30106	2.111	274.37		
	stdb2				33487	63203	1.887			

Appendix 2 GC/MS Results from the Analysis of the Tree Samples

Extraction Date	Highway	Mile	Tree #	Volume Sample ml	Peak Areas Internal Standard	Peak Areas HCB	HCB / Int Std	[HCB] ppb		Standard Average HCB/INT STD
	stda1				11175	20484	1.833			1.857
12/10/97	Seward	75	2a	0.4280	5413	254	0.047	6.32		
1/29/98	Ak	1260	1		12416	710	0.057	7.70		
11/19/97	Ak	1301	1b	0.3280	6691	268	0.040	5.39		
11/18/97	Dalton	160	2b	0.2750	7034	1471	0.209	28.16		
1/29/98	Rich	31	2a	0.3300	6812	299	0.044	5.91		
	stda2				9442	17555	1.859			
	stdb1				9320	17518	1.880			
12/12/97	Rich	309	1-spike	0.4400	4458	14001	3.141	422.88		
1/29/98	Blank 1-29			0.3290	6919	0	0.000	0.00		
1/29/98	Rich	339	1b	0.3700	6026	395	0.066	8.83		
11/26/97	Rich	109	2	0.2800	6255	303	0.048	6.52		
12/10/97	Rich	339	2b	0.2830	6694	391	0.058	7.86		
	stdb2				8651	16047	1.855			

Appendix 3 Lipid and Water Data

Highway	Mile	Tree #	Needle Mass	Water Mass	% Water	Total Lipid	% Lipid
Ak	1260	1	9.933	4.640	46.711		
Ak	1418	2-spike	9.944	5.036	50.648	0.150	1.512
Ak	1418	1	9.883	4.835	48.926	0.176	1.783
Ak	1301	1a	9.998	5.225	52.257	0.106	1.063
Ak	1301	2a	9.993	4.981	49.849	0.107	1.068
Ak	1301	2b	9.978	4.974	49.849	0.108	1.082
Ak	1230	1-spike	9.821	4.999	50.899	0.179	1.826
Ak	1230	1	9.974	5.077	50.899	0.183	1.838
Ak	1384	2	9.990	4.913	49.184	0.078	0.781
Ak	1384	1	9.970	4.932	49.464	0.107	1.071
Ak	1230	2-spike	9.916	5.106	51.497	0.156	1.572
Ak	1230	2	9.933	5.115	51.497	0.141	1.423
Ak	1260	2	9.978	5.007	50.183	0.490	4.913
Ak	1418	1-spike	9.917	4.852	48.926	0.180	1.812
Ak	1345	2	9.783	4.919	50.281	0.080	0.815
Ak	1260	1	10.009	4.675	46.711	0.320	3.194
Ak	1301	1b	9.964	5.207	52.257	0.086	0.861
Ak	1345	1	9.990	5.180	51.847	0.098	0.980
Ak	1418	2	9.999	5.064	50.648	0.145	1.454
Dalton	193	2-spike	9.925	4.778	48.143	0.128	1.288
Dalton	193	1-spike	9.973	5.265	52.793	0.218	2.190
Dalton	122	1	9.971	4.949	49.630	0.363	3.645
Dalton	50	2	9.985	5.071	50.783	0.329	3.298
Dalton	193	1	9.958	5.257	52.793	0.146	1.470
Dalton	13	2b	10.008	5.142	51.375	0.129	1.286
Dalton	13	2a	9.989	5.132	51.375	0.140	1.404
Dalton	160	2a	9.977	4.668	46.787	0.181	1.817
Dalton	50	1	9.904	5.080	51.288	0.384	3.877
Dalton	122	2	9.937	5.041	50.733	0.307	3.087
Dalton	87	2	9.949	5.100	51.260	0.120	1.202
Dalton	50	1-spike	9.928	5.090	51.270	0.392	3.952
Dalton	193	2	9.927	4.779	48.143	0.131	1.317
Dalton	160	1b	9.950	5.013	50.385	0.254	2.550
Dalton	13	1a	9.987	5.016	50.225	0.169	1.694
Dalton	13	1b	9.985	5.015	50.225	0.184	1.844
Dalton	160	1a	9.926	5.001	50.385	0.258	2.595
Dalton	87	1	9.867	5.407	54.800	0.166	1.686
Dalton	50	2-spike	9.945	5.059	50.873	0.330	3.318
Dalton	160	2b	9.986	4.672	46.787	0.202	2.026
Elliot	47	2	9.890	4.875	49.288	0.321	3.245
Elliot	47	2-spike	9.970	4.914	49.288	0.320	3.207
Elliot	9	1b	9.961	5.189	52.095	0.106	1.067
Elliot	9	1a	9.986	5.202	52.095	0.122	1.223
Elliot	9	2b	9.978	5.004	50.147	0.189	1.896
Elliot	47	1-spike	9.887	4.677	47.304	0.337	3.412
Elliot	47	1	9.966	4.714	47.304	0.345	3.459
Elliot	9	2a	10.001	5.015	50.147	0.170	1.697

Appendix 3 Lipid and Water Data

Highway	Mile	Tree #	Needle Mass	Water Mass	% Water	Total Lipid	% Lipid
Goldstream		1	9.995	5.059	50.620	0.175	1.756
Goldstrean		2	9.926	4.801	48.367	0.161	1.625
Parks	67	2	9.902	5.409	54.630	0.097	0.981
Parks	147	2a	9.958	5.128	51.495	0.122	1.222
Parks	230	1-spike	9.851	4.972	50.472	0.170	1.726
Parks	107	1-spike	9.689	4.782	49.357	0.070	0.723
Parks	270	2a	9.984	4.942	49.504	0.419	4.196
Parks	230	1	9.896	4.995	50.472	0.176	1.778
Parks	270	2b	9.963	4.932	49.504	0.429	4.308
Parks	147	1a	9.976	5.388	54.014	0.073	0.736
Parks	230	2	9.805	5.057	51.578	0.138	1.407
Parks	270	1a	9.867	4.976	50.431	0.346	3.505
Parks	187	2	9.995	4.637	46.394	0.114	1.141
Parks	214	2	9.932	4.806	48.391	0.146	1.474
Parks	147	2b	9.923	5.110	51.495	0.123	1.237
Parks	214	1	9.896	5.182	52.368	0.147	1.490
Parks	230	2-spike	9.916	5.114	51.578	0.132	1.331
Parks	270	1b	9.964	5.025	50.431	0.359	3.601
Parks	67	1	9.922	5.325	53.670	0.097	0.978
Parks	147	1b	9.947	5.373	54.014	0.078	0.783
Parks	107	1	9.998	4.935	49.357	0.085	0.846
Parks	107	2	9.904	4.922	49.695	0.256	2.585
Parks	107	2-spike	9.992	4.965	49.695	0.264	2.639
Parks	187	1	9.965	5.099	51.172	0.098	0.985
Rich	339	1a	9.935	4.647	46.778	0.277	2.785
Rich	166	1-spike	9.919	5.021	50.617	0.167	1.682
Rich	166	1	9.841	4.981	50.617	0.172	1.751
Rich	4	2	10.015	5.285	52.768	0.167	1.666
Rich	166	2	10.003	4.958	49.567	0.302	3.023
Rich	309	2	9.904	4.773	48.197	0.159	1.608
Rich	277	1	9.878	5.262	53.272	0.107	1.085
Rich	16	1-spike	9.993	5.208	52.120	0.173	1.736
Rich	16	1	9.918	5.169	52.120	0.173	1.749
Rich	309	1	9.957	5.202	52.245	0.123	1.232
Rich	339	2a	9.962	4.537	45.542	0.166	1.662
Rich	31	1a	9.965	5.076	50.937	0.123	1.231
Rich	31	1b	9.978	5.083	50.937	0.974	9.763
Rich	277	2	9.946	5.122	51.494	0.146	1.464
Rich	72	2	9.940	4.878	49.076	0.160	1.614
Rich	4	1	10.014	5.064	50.571	0.248	2.481
Rich	16	2	10.004	5.125	51.231	0.174	1.735
Rich	31	2b	9.988	4.983	49.889	0.104	1.041
Rich	309	2-spike	9.945	4.793	48.197	0.153	1.534
Rich	16	2-spike	10.005	5.126	51.231	0.163	1.633
Rich	207	2b	9.963	5.159	51.782	0.145	1.450
Rich	109	1	10.007	5.298	52.944	0.194	1.943
Rich	207	2a	9.929	5.141	51.782	0.143	1.444
Rich	72	1	9.990	4.850	48.551	0.220	2.203

Appendix 3 Lipid and Water Data

Highway	Mile	Tree #	Needle Mass	Water Mass	% Water	Total Lipid	% Lipid
Rich	166	2-spike	9.998	4.956	49.567	0.284	2.843
Rich	31	2a	9.939	4.959	49.889	0.099	0.993
Rich	309	1-spike	9.955	5.201	52.245	0.127	1.275
Rich	339	1b	9.949	4.654	46.778	0.278	2.799
Rich	109	2	10.005	5.022	50.194	0.114	1.138
Rich	339	2b	9.981	4.546	45.542	0.157	1.569
Seward	8	2a	10.009	5.449	54.439	0.167	1.670
Seward	8	2b	9.943	5.413	54.439	0.193	1.942
Seward	8	1b	9.912	5.034	50.784	0.234	2.361
Seward	8	1a	9.935	5.045	50.784	0.251	2.529
Seward	42	1-spike	9.879	5.253	53.170	0.171	1.728
Seward	75	2a	9.760	5.115	52.404	0.098	1.000
Seward	42	2	9.955	5.130	51.529	0.204	2.047
Seward	42	1	9.940	5.285	53.170	0.159	1.597
Seward	42	2-spike	10.004	5.155	51.529	0.199	1.992

Appendix 4 Percent Recovery of the Internal Standard

Extraction Date	Highway	Mile	Tree #	Internal Standard Area	Average Area of Internal Standard In Standards	Sample Volume	% Recovery of Internal Standard
3/3/98	Ak	1230	1-spike	15285	25072	0.384	93.6
3/3/98	Ak	1230	1	12978	25072	0.342	70.8
3/3/98	Ak	1230	2-spike	16493	33005	0.285	57.0
3/3/98	Ak	1230	2	18893	33005	0.288	65.9
2/13/98	Ak	1260	1	0	14137	0.370	0.0
11/3/97	Ak	1260	2	0	11570	0.410	0.0
1/29/98	Ak	1260	1	12416	9647		
11/18/97	Ak	1301	1a	6551	10905	0.260	62.5
11/19/97	Ak	1301	2a	1183	13501	0.320	11.2
11/19/97	Ak	1301	2b	8636	13501	0.280	71.6
11/19/97	Ak	1301	1b	6691	9647	0.328	91.0
3/11/98	Ak	1345	2	17396	31999	0.375	81.5
2/26/98	Ak	1384	2	17120	25072	0.270	73.7
2/26/98	Ak	1384	1	14949	33005	0.324	58.7
2/12/98	Ak	1418	2-spike	6168	14137	0.390	68.1
2/3/98	Ak	1418	1	11767	14137	0.230	76.6
2/3/98	Ak	1418	1-spike	15282	21432	0.344	98.1
3/11/98	Ak	1345	1	14033	21886	0.255	65.4
2/12/98	Ak	1418	2	10537	12864	0.255	83.5
11/18/97	Blank 11/18			10590	11570	0.280	102.5
11/19/97	Blank 11-19			18319	13501	0.155	84.1
11/26/97	Blank 11-26			6126	10905		
12/10/97	Blank 12-10			7799	10905		
12/12/97	Blank 12-12			7017	14137	0.335	66.5
1/29/98	Blank 1-29			6919	9647	0.329	94.4
2/11/98	Blank 2/11			6118	12864	0.352	67.0
2/12/98	Blank 2/12			8067	12864	0.312	78.3
2/18/98	Blank 2/12			840	12864	0.425	11.1
2/26/98	Blank 2/26			10332	26173	0.350	55.3
2/3/98	Blank 2/3			9636	21432	0.460	82.7
2/13/98	Blank 2-13			13768	13501	0.252	102.8
2/20/98	Blank 2-20			7880	14137	0.400	89.2
3/10/98	Blank 3/10			29110	31999	0.205	74.6

Appendix 4 Percent Recovery of the Internal Standard

Extraction Date	Highway	Mile	Tree #	Internal Standard	Average Area of Internal Standard In Standards	Sample Volume	% Recovery of Internal Standard
3/11/98	Blank 3/11			12826	31999	0.365	58.5
3/3/98	Blank 3-3			18246	25072	0.250	72.8
4/7/98	blank 4/7			0	28791	0.337	0.0
4/7/98	Blank 4/7			14341	21886	0.288	75.5
12/10/97	Dalton	13	2b	7440	10905	0.280	76.4
12/10/97	Dalton	13	2a	8501	13501	0.355	89.4
11/3/97	Dalton	13	1a	8816	11570	0.295	89.9
11/3/97	Dalton	13	1b	8577	11570	0.225	66.7
3/10/98	Dalton	50	2	11897	21886	0.265	57.6
3/3/98	Dalton	50	1	12504	25072	0.360	71.8
3/3/98	Dalton	50	1-spike	13032	33005	0.292	46.1
3/10/98	Dalton	50	2-spike	14295	31999	0.200	35.7
3/3/98	Dalton	87	2	20061	25072	0.162	51.8
2/18/98	Dalton	87	1	11394	21432	0.338	71.9
3/11/98	Dalton	122	1	8044	21886	0.375	55.1
3/3/98	Dalton	122	2	17719	25072	0.162	45.8
11/18/97	Dalton	160	2a	12078	13501	0.180	64.4
11/19/97	Dalton	160	1b	9299	11570	0.300	96.4
11/19/97	Dalton	160	1a	8910	11570	0.330	101.7
11/18/97	Dalton	160	2b	7034	9647	0.275	80.2
2/13/98	Dalton	193	2-spike	11354.5	14137	0.272	87.4
2/13/98	Dalton	193	1-spike	13787.5	14137	0.222	86.6
2/12/98	Dalton	193	1	10718	12864	lost	
2/13/98	Dalton	193	2	21265	26173	0.238	77.3
11/26/97	Elliot	9	1b	5425	10905	0.390	77.6
11/26/97	Elliot	9	1a	8983	11570	0.415	128.9
2/11/98	Elliot	9	2b	15154	21432	0.262	74.1
2/11/98	Elliot	9	2a	12619	21432	0.340	80.1
4/7/98	Elliot	47	2	7418	28791	0.280	28.9
4/7/98	Elliot	47	2-spike	10876	28791	0.290	43.8
2/3/98	Elliot	47	1-spike	21170	21432	0.342	135.1
2/3/98	Elliot	47	1	0	21432	0.210	0.0
4/7/98	Goldstream		1	8454	28791	0.321	37.7

Appendix 4 Percent Recovery of the Internal Standard

Extraction Date	Highway	Mile	Tree #	Internal Standard	Average Area of Internal Standard In Standards	Sample Volume	% Recovery of Internal Standard
4/7/98	Goldstrean		2	11022	28791	0.470	72.0
2/11/98	Parks	67	2	13459	14137	0.245	93.3
2/3/98	Parks	67	1	15750	21432	0.344	101.1
3/11/98	Parks	107	1-spike	10310	21886	0.450	84.8
3/11/98	Parks	107	1	20438	31999	0.310	79.2
3/10/98	Parks	107	2	12842	31999	0.340	54.6
3/10/98	Parks	107	2-spike	12733	31999	0.330	52.5
2/12/98	Parks	147	2a	9784	14137	0.291	80.6
2/11/98	Parks	147	1a	6730	10905	0.345	85.2
2/12/98	Parks	147	2b	14478	26173	0.304	67.3
2/11/98	Parks	147	1b	10897	21432	0.368	74.8
3/11/98	Parks	187	2	23718	33005	0.335	96.3
3/11/98	Parks	187	1	16213	31999	0.350	70.9
2/26/98	Parks	214	2	17934	33005	0.286	62.2
2/26/98	Parks	214	1	14073	26173	0.295	63.4
4/7/98	Parks	230	1-spike	10050	28791	0.450	62.8
4/7/98	Parks	230	1	13640	21886	0.294	73.3
2/26/98	Parks	230	2	15129	25072	0.272	65.7
2/26/98	Parks	230	2-spike	11846	26173	0.388	70.2
4/7/98	Parks	270	2a	6142	21886	0.330	37.0
4/7/98	Parks	270	2b	7500	21886	0.256	35.1
2/26/98	Parks	270	1a	11163	25072	0.255	45.4
2/26/98	Parks	270	1b	13044	26173	0.392	78.1
3/11/98	Rich	4	2	15882	21886	0.250	72.6
3/10/98	Rich	4	1	11858	33005	0.375	53.9
2/18/98	Rich	16	1-spike	6082.5	10905	0.385	85.9
2/18/98	Rich	16	1	11408	10905	0.379	158.6
2/26/98	Rich	16	2	27547	26173	0.210	88.4
2/26/98	Rich	16	2-spike	11404	26173	0.368	64.1
12/12/97	Rich	31	1a	10258	13501	0.259	78.7
12/12/97	Rich	31	1b	6576	13501	0.385	75.0
1/29/98	Rich	31	2b	23220	26173	0.280	99.4

Appendix 4 Percent Recovery of the Internal Standard

Extraction Date	Highway	Mile	Tree #	Internal Standard	Average Area of Internal Standard In Standards	Sample Volume	% Recovery of Internal Standard
1/29/98	Rich	31	2a	6812	9647	0.330	93.2
3/3/98	Rich	72	2	20329	33005	0.244	60.1
3/10/98	Rich	72	1	13318	31999	0.375	62.4
11/3/97	Rich	109	1	9343	11570	0.350	113.1
11/26/97	Rich	109	2	6255	9647	0.280	72.6
4/7/98	Rich	166	1-spike	11104	28791	0.449	69.3
4/7/98	Rich	166	1	6486	28791	0.645	58.1
3/11/98	Rich	166	2	13865	21886	0.260	65.9
3/11/98	Rich	166	2-spike	14263	31999	0.275	49.0
11/26/97	Rich	207	2b	16155	15125	0.310	132.4
11/26/98	Rich	207	2a	24860	15125	0.265	174.2
2/20/98	Rich	277	1	6245	12864	0.400	77.7
2/26/98	Rich	277	2	13722	33005	0.362	60.2
2/20/98	Rich	309	2	8692	12864	0.362	97.8
12/12/97	Rich	309	1	6605	10905	0.348	84.3
2/20/98	Rich	309	2-spike	18007.5	26173	0.255	70.2
12/12/97	Rich	309	1-spike	4458	9647	0.440	81.3
2/13/98	Rich	339	1a	12239	14137	0.253	87.6
12/10/97	Rich	339	2a	11675	13501	0.250	86.5
1/29/98	Rich	339	1b	6026	9647	0.370	92.4
12/10/97	Rich	339	2b	6694	9647	0.283	78.5
11/3/97	Seward	8	2a	4764	10905	0.410	71.6
11/3/97	Seward	8	2b	10875	13501	0.320	103.1
3/3/98	Seward	8	1b	11532	25072	0.310	57.0
3/3/98	Seward	8	1a	20531	33005	0.254	63.2
2/20/98	Seward	42	1-spike	10604.5	21432	0.320	63.3
12/10/97	Seward	75	2a	5413	9647	0.428	96.1
2/18/98	Seward	42	2	7866	12864	0.385	94.2
2/20/98	Seward	42	1	6274	12864	0.380	74.1
2/18/98	Seward	42	2-spike	9150.5	12864	0.240	68.3